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RELATION OF OXYGEN PRESSURE AND TEMPERATURE TO THE INFLUENCE OF ETHYLENE ON CARBON-DIOXIDE PRODUCTION AND ON SHOOT ELONGATION IN VERY YOUNG WHEAT SEEDLINGS[1]

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(WITH FIVE FIGURES)

Ethylene influence on plant processes; review of literature

That illuminating gas is injurious and often fatal to plants was reported by GIRARDIN (**16**) in 1864. Other observers, among whom were NELJUBOW (**34, 35**), KNY (**28**), VIRCHOW (**57**), SPÄTH and MEYER (**51**), MOLISCH (**33**), STONE (**52, 53**), and WILCOX (**62**), described symptoms of plants injured by illuminating gas or laboratory air. Some of the more striking symptoms described were wilting, yellowing, and falling of leaves; browning and death of cambium in trunks of shade trees and conspicuous fungous growth on injured trunks; increased diameter and characteristic bending of roots of maize seedlings; formation of enlarged lenticels on willow twigs in humid atmosphere; and loss of negative geotropism in stems of certain seedlings, including wheat. CROCKER and KNIGHT (**4**) discovered that minute traces of illuminating gas in the atmosphere prevented the opening of carnation flower buds, and demonstrated that ethylene was the most active toxic agent in causing this injury.

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CROCKER, KNIGHT, and ROSE (5) showed that very small concentrations of ethylene in the air might produce characteristic reactions, particularly in etiolated seedlings of sweet pea. In an atmosphere containing one volume of ethylene in a million of air, etiolated sweet pea seedlings had thicker and shorter stems than other like seedlings that had not been exposed to ethylene, and the affected seedlings failed to exhibit the usual negative geotropism of epicotyls. KNIGHT and CROCKER (26) noted similar effects of tobacco smoke and attributed these effects to ethylene contained in the smoke. HARVEY and ROSE (20) discovered that seedling roots of species of *Hibiscus*, *Syringa*, *Ailanthus*, *Croton*, *Diervilla*, *Ricinus*, and *Ulmus*, when treated with ethylene, showed sloughing off of the cork tissue and great proliferation of other tissues from the phloem outward. HARVEY (19) found that sweet pea seedlings injured by ethylene contained relatively larger amounts of simple soluble compounds and relatively smaller amounts of more complex soluble compounds and insoluble ones than were present in similar seedlings that had not been exposed to ethylene. The osmotic values and the permeability of ethylene-treated tissues were somewhat greater than those of untreated tissues and respiration was somewhat less rapid, except for a temporary acceleration of CO_2 production that occurred during the first three hours of exposure to ethylene.

SORAUER (50) confirmed earlier reports that treatment with illuminating gas might cause leaf yellowing, leaf fall, and failure of flower buds to open, but he attributed these effects to inadequate oxygen supply. WEHMER (61) observed similar effects of illuminating gas acting as a poison, and found that the stage of development of the plant and its state of health were important in determining the extent of the gas influence. DOUBT (13) observed that plants of *Coleus*, *Ricinus*, *Datura*, and *Mimosa* lost their leaves after having been exposed to illuminating gas for a few hours. *Mimosa* lost its sensitiveness to touch before other forms of injury were pronounced. She found also that illuminating gas or ethylene caused proliferation of twigs of apple and pear, especially in lenticels and leaf scars. GOODSPEED, MCGEE, and HODGSON (17), and also KENDALL (25), reported that CO, CO_2, and ethylene (all commonly present in il-

luminating gas) each caused premature abscission of flowers and fruits in forms of *Nicotiana* and *Citrus*. SCHWARTZ (**47**) confirmed reports of DOUBT and other earlier observers that petioles of *Helianthus*, *Lycopersicum*, and *Salvia* showed epinastic curvatures in the presence of illuminating gas.

WALLACE (**58, 59, 60**) investigated internal and external conditions influencing the formation of intumescences on apple twigs in the presence of ethylene, and observed that air humidity had no influence in his experiments, but that temperature and oxygen content of the atmosphere were influential. Between the limits of 75% of ethylene and 25% of oxygen at one extreme and one part of ethylene in 10,000 of air at the other, the concentration of ethylene made little difference in the nature or degree of the effects produced; concentrations as low as one volume of ethylene in 100 million volumes of air caused some formation of intumescences.

PRIESTLEY (**37, 38**) pointed out that etiolated pea seedlings have in health a well-marked Casparian strip in the primary endodermis, extending from the base of the stem nearly to the stem apex, but that this strip failed to form under the influence of ethylene. He suggested that this influence of ethylene might be due to increased mobility of unsaturated fatty acids.

The commercial use of ethylene to hasten the advent of an edible condition in fruits and vegetables and to shorten the dormant period in resting plant organs has been recently developed from the work of many experimenters. CHACE and DENNY (**1**) reported that the yellowing of citrus fruits could be accelerated by exposing them to an atmosphere of one volume of ethylene in 5000 or more volumes of air. To hasten the blanching of celery, HARVEY (**22**) recommended the use of one volume of ethylene in from 1000 to 10,000 volumes of air; he suggested later (**23**) that the same treatment might be used to accelerate ripening in bananas, pineapples, and other tropical fruits. ROSA (**43**) found that ethylene hastened the ripening of tomatoes, and he afterwards (**44**) reported that propylene was more effective. CHACE and SORBER (**3**) recommended the use of ethylene to promote the softening of Bartlett pears; and CHACE and CHURCH (**2**) and DAVIS and CHURCH (**6**) found that ethylene treatment hastened coloring, softening, and loss of astringency in persimmons.

McCallum (**30**) was able to shorten the period of dormancy of potato tubers by brief treatment with ethylene compounds and related substances. Rosa (**45, 46**) found that potato tubers that had been treated with ethylene sprouted earlier and produced an unusually large number of sprouts from each eye. Vacha and Harvey (**56**) observed that a 6-day exposure to one volume of ethylene in 1000 volumes of air caused more rapid sprouting in Bliss Triumph and Russet Burbank potato tubers, but that other potato varieties tested by them were not perceptibly affected. They found also that ethylene, and propylene also, hastened the sprouting of gladiolus corms and quickly broke the dormancy of hardwood cuttings of apple, pear, plum, cherry, mock-orange, and honeysuckle. Both gases accelerated the germination of seeds of buckthorn, high-bush cranberry, Tartarian honeysuckle, and snowberry. Haber (**18**) reported that a 24-hour exposure of onion sets to ethylene-air mixtures containing one part of ethylene to 100 or 200 parts of air accelerated early growth and resulted in unusually large bulbs. Narcissus bulbs matured earlier when treated with an ethylene-air mixture of one part of ethylene to 200 or 300 parts of air. Air humidity appeared to be influential, for the lower concentration of ethylene was as effective with high relative humidity as the higher concentration was with very low relative humidity. Haber stated that when sweet-corn kernels were soaked for 24 or 48 hours in a solution formed by bubbling ethylene through water, they germinated more rapidly than similar seeds soaked in untreated water for the same length of time and at the same temperature. Plants from ethylene-treated seeds produced edible ears earlier than did those from untreated seeds.

Not all investigators have found ethylene to be uniformly effective to break dormancy or hasten the processes of ripening and maturing in plant organs. Earlier experiments of Chace and Church (**2**) showed but little effect from the use of ethylene for hastening the ripening of tomatoes; but their later experiments, in which degree of maturity of the fruits was taken into account, showed relatively small but notable effects. They found also that ethylene treatment did not alter the percentage of moisture, reducing sugars, sucrose, citric acid, or pentosans in citrus fruits, although it did accelerate

coloring. Treatment of avocados with ethylene caused the development of a brassy color of the skin and a darkening of the flesh. WORK (**63**) observed but little effect of ethylene in the ripening of tomatoes, excepting that fruits in the green-mature stage ripened more promptly after treatment with this gas. ROBBINS (**42**) found no advantage in the use of ethylene to hasten the blanching of celery, either in a tight compartment or in a refrigerator car. He also reported that ethylene treatment gave no acceleration of ripening in Marglobe and Greater Baltimore tomatoes with temperature ranges of 55°–65°, 68°–70°, or 70°–75° F. KOHMAN (**29**) reported that ethylene treatment was of no value for treating tomatoes to be used for canning. DENNY (**9, 10**) found that ethylene was ineffective in terminating the dormancy of potato tubers, with air mixtures containing ethylene at concentrations between 5 and 150,000 volumes per million volumes of air. He reported (**12**) that exposure for 12 days to a 1:1000 ethylene-air mixture failed to shorten the rest period of freshly harvested gladiolus corms, although this treatment was effective on corms one month after harvest. Similarly, ethylene treatment was ineffective in breaking the dormancy of pot-grown lilacs and crabapples (**11**), although it was effective on forms with less pronounced dormancy, such as *Prunus triloba*. DENNY observed also (**7**) that little or no acceleration of the yellowing process occurred in lemons at temperatures below 45° or above 92° F., or if the oxygen concentration were very low or the ethylene concentration were above 80%. Although HIBBARD (**24**) found that ethylene hastened destruction of chlorophyll in celery, oranges, and apples, he did not recommend its use for hastening the blanching of celery or for ripening bananas. FULTON, STEVENS, and WOOTTEN (**15**) stated that oranges should be nearly mature for ethylene treatment to be effective; with immature fruit, too high temperature, or too great concentration of ethylene, serious damage might result from ethylene treatment.

Several of the studies just mentioned dealt to some extent with physiological questions other than those of immediate practical interest. DENNY (**8**) reported that treatment with ethylene at concentrations of from one to 1000 volumes in a million volumes of air caused increases of from 100 to 250% in the rate of CO_2 production

by lemons. With bananas, REGEIMBAL, VACHA, and HARVEY (**40**) observed increases in the rate of CO_2 production as great as 150% within 15 or 20 minutes after the fruits were exposed to a mixture of ethylene and air containing one volume of ethylene in a thousand. This remarkable acceleration disappeared entirely in half an hour if no more ethylene was introduced into the respiration chamber, but a second and third dose of ethylene resulted in similar brief outbursts. The same investigators reported that bananas ripened with ethylene contained 20–25% more sugar than those ripened by ordinary methods. MACK (**31**) observed increased CO_2 production and more rapid decay in celery plants treated with ethylene than in otherwise similar but untreated plants. DAVIS and CHURCH (**6**) found that CO_2 production by ripening persimmons was accelerated by exposure to a gas mixture of one volume of ethylene to 1000 volumes of air.

REGEIMBAL and HARVEY (**41**) observed that activity of the proteolytic enzym bromelin was somewhat greater in juice from ethylene-ripened pineapples than in juice from untreated fruits, but the treated pineapples were said to have been somewhat riper than the others at the time treatment was applied, and it is at least possible that this difference in bromelin activity may not have been due to ethylene treatment. ENGLIS and ZANNIS (**14**) found that ethylene had no effect on the activity of diastase and invertase in their experiments. REA and MULLINIX (**39**) reported that exposure to ethylene caused maize and wheat starch to be transformed into sugar, both in the dry state and in suspensions containing 1 gm. of starch in 100 ml. of water.

From this incomplete review it is clear that our present knowledge of ethylene influence on plants and plant substances is so fragmentary that satisfactory general statements about ethylene relations are not yet possible. There seems to be no doubt that ethylene has sometimes produced stimulation and sometimes retardation, and that it has sometimes failed to exert any notable influence on the processes considered. Experimentation has been predominantly of a preliminary and necessarily somewhat naïve nature, and most of the experimental results that have been published must be considered as merely isolated facts, many of which appear to be discordant. Constructive comparisons are generally impossible, either

because the plant material employed for different tests has differed in unknown ways or because the experimental procedure has been such as to preclude satisfactory repetition and comparison. A considerable part of the experimentation in this field has been planned with reference to technological aims, with a desire to establish commercially useful methods for ripening fruits, awakening dormant tissues, and the like, rather than to advance fundamental knowledge. An apparent desire to encourage the use of ethylene in the commercial handling of horticultural products is detectable in some recent publications on ethylene effects, but that does not necessarily detract from the scientific value of the recorded observations. On the other hand, discrepant results apparently due to fundamentally unsatisfactory logical or experimental technique not only tend to retard practical applications but also do not, in themselves, greatly accelerate the advance of scientific knowledge.

The general subject of chemical stimulation and toxicity occupies a prominent position in plant physiology and its many applications. Consistently and adequately planned experimental studies in this field, which are becoming relatively more feasible as the concept of multiple causation or determination becomes more widely appreciated, are greatly needed to advance our scientific grasp of the control of vital processes in general. The number of chemical compounds that call for consistent investigation in this connection is great, for almost every compound would doubtless exert some influence if applied to plants in suitable concentrations or at suitable rates of supply, with adequately chosen time periods and background complexes. Aside from simple convenience with regard to experimental technique, it would make little difference what compound or compounds might be selected for studies of this sort. Ethylene, however, lends itself rather readily to experimentation with several experimental variables, and it is clear that results secured from experiments with this substance may throw light on questions of horticultural importance as well as on important aspects of physiological science.

Preliminary experimentation

The studies reported in this paper were an outgrowth from a few practical tests concerning the value of ethylene as an agent for

hastening the blanching of celery, as recommended by HARVEY (**22**). They were begun as a series of preliminary experiments planned for orientation. Some of the results with celery have been reported previously (**31**) and others will be presented later in this paper. Additional preliminary experiments were also performed with material other than celery. Many valuable suggestions were received from Professor D. E. HALEY of the Pennsylvania State College, where the preliminary work was done.

The present section is devoted to the preliminary experiments and the following section reports a more elaborate study, on wheat seedlings.

In the first celery tests, some of the results of which have been reported (**31**), galvanized sheet-iron cylinders with a capacity of about 120 liters were used as containers. Each was made gas-tight by means of a broad rubber band fitting tightly around the top and partly over the edge of the lid. Gases were admitted or withdrawn by means of two metal tubes soldered into holes in each lid, the tubes being ordinarily closed by means of short pieces of rubber tubing and screw cocks. At the beginning of each experiment one, two, or three celery plants were inclosed in each container, in an atmosphere of ordinary air, and a volume of commercial ethylene sufficient to make up the desired initial gas concentration was then admitted from a gas burette. The volume of ethylene required was always so small that it could be added to the container without appreciable change in total gas pressure.

Observations on the rate of blanching were made from time to time through a glass plate which closed a suitable opening in the lid of one of the containers, and was ordinarily covered to exclude light. Final observations were made after removing the plants from all containers. Estimates of the average hourly rates of CO_2 production were made from measurements of the total amount of CO_2 that had accumulated in each container during the experimental period. These measurements were made by sweeping out the accumulated CO_2 with a rapid stream of CO_2-free air, absorbing the CO_2 in soda-lime and weighing.

In these experiments, blanching was accelerated by ethylene in concentrations varying between one volume and 100 volumes in 50,000

volumes of air. Most rapid blanching occurred with the lower concentrations of ethylene. With the higher concentrations injury of the petioles was observed, splitting and peeling of the inner surface and development of pithiness. Decay occurred in many instances, its progress being most rapid with the lower concentrations of ethylene, which produced most rapid blanching. Carbon-dioxide production was most rapid when blanching and decay were most pronounced.

Additional preliminary experiments that are to be reported at this time dealt with the effect of ethylene on the catalase activity of celery plants, with immediate effects of ethylene on CO_2 production by celery and chard leaves, tomato fruits, and wheat seedlings, and with the influence of ethylene on catalase activity and oxidase activity in wheat seedlings.

EFFECTS OF ETHYLENE ON CATALASE ACTIVITY OF CELERY.—For the catalase tests, celery plants were sealed in separate containers, as already described, for periods of from 30 min. to 48 hr. At the start of each test the control chambers contained ordinary air and the others contained known mixtures of air and ethylene. Before each plant was placed in its chamber a sample of leaf tissues was taken and a similar sample was taken at the end of the test, and catalase activity was estimated for each sample. A sample consisted of five discs cut from a leaf by means of a 1-cm. cork borer. The five discs cut before placing the plants in their containers were taken from as many different leaflets of the same leaf, and those cut after treatment were from the same leaflets as had been used before, the two cuts being symmetrically placed on each side of a conspicuous vein. The five discs of each sample were ground, with their weight of powdered $CaCO_3$, in 50 times their weight of distilled water, and the preparation was stored in a rubber-stoppered bottle for 24 hr. in an ordinary refrigerator. KNOTT (**27**) reported that this method of preparing leaf samples gave consistent results for catalase activity.

Relative catalase activity was measured according to the method described by KNOTT (**27**). Two ml. of leaf-tissue infusion were shaken with 2 ml. of standard H_2O_2 solution ("Dioxogen") neutralized with powdered $CaCO_3$. The oxygen produced was measured at atmospheric pressure in a eudiometer and relative catalase activity was taken to be inversely proportional to the time required to produce a

standard volume of oxygen. Only small changes in catalase activity were found to have occurred during any of the experimental periods, and such changes as did occur were apparently not related to the concentration of ethylene at the start of the experiment; they may have been associated with small temperature differences. At any rate, no effect of ethylene on catalase activity was apparent.

IMMEDIATE EFFECTS OF ETHYLENE ON CO_2 PRODUCTION.—In the experiments on the immediate effects of ethylene, 4-liter tubulated bell-jars were used as experiment chambers. The bottom of each jar, through which the plant material was introduced, was closed by means of a circular glass plate held tightly in position by means of a broad rubber band. Each jar was provided with a rubber stopper bearing an inlet and an outlet tube, the inlet projecting only slightly below the stopper while the outlet extended nearly to the bottom of the jar. Each inlet tube forked just above the stopper, one branch leading from a series of wash bottles with KOH solution for the removal of CO_2 from the incoming air, while the other branch (provided with a glass stopcock for regulating delivery of ethylene) led to a reservoir of ethylene under slight pressure. The stopper was provided with several additional tubes, each closed by means of rubber tubing and screw cock, so that the gaseous contents of the jar might be confined or withdrawn at will, or a current of air might be passed continuously through the jar by means of a filter pump. Each outlet tube led to a Pettenkofer tube partly filled with glass beads and containing a measured amount of standard $Ba(OH)_2$ solution, for absorbing the CO_2 given off by the plant material. During an experiment each jar was surrounded by water in a reservoir from which light was excluded. The water provided a seal and served to regulate the temperature.

At the beginning of each experiment the container was sealed for 15 or 25 min. and then the gas contents were swept out by means of a rapid current of air continuing for 5 min. Thus the CO_2 evolved by the plant material in 20 or 30 min. was removed and absorbed in the Pettenkofer tube, being finally measured by titration of the $Ba(OH)_2$ solution with standard HCl. At the end of this 5-min. period the bell-jar was again sealed and the procedure repeated. Several successive measurements of the amount of CO_2 produced in 20 or in 30

min. were made in this manner, until a fairly constant rate of respiration was indicated. Ethylene was then introduced, in quantity sufficient to make the volumetric proportion of ethylene to air in the container either 1:400 or 1:800, and measurements of CO_2 production were continued as before. Exposure to ethylene was for only one period at a time, although the same material was treated with ethylene at several different times, usually separated by intervals greater than 1 hr.

Experiments of this sort were performed with celery and chard leaves and with tomato fruits. The rate of evolution of CO_2 was found to fluctuate somewhat from period to period but its fluctuations were apparently not related to the ethylene treatment in any consistent way. In some instances the rate was higher with ethylene than without it and in other instances it was lower with ethylene. With these materials, and with the procedure followed in these tests, the respiration rate was apparently not influenced by a 15-min. or a 25-min. exposure of the material to an ethylene-air mixture containing 400 or 800 volumes of air to one volume of ethylene.

PRELIMINARY EXPERIMENTS ON CELERY WITH MAINTAINED GASEOUS ENVIRONMENTS.—In the experiments already described the composition of the atmospheric environment was known only for the very beginning of an exposure. Changes in the oxygen and CO_2 content of the atmosphere must occur in experiments of this kind, becoming more pronounced as the exposure period is prolonged, and it is probable that the concentration of ethylene in the inclosed space decreases with time. With this probability in mind a method was adopted by which a known mixture of ethylene and CO_2-free air was passed continuously through the experiment chamber, so that the composition of the inclosed atmosphere was maintained practically constant throughout the whole period of exposure. All the CO_2 given off was absorbed, being measured at intervals, and the experimental period was prolonged until changes in the appearance of the plant material were clearly evident.

The apparatus employed was much like that described in the preceding section. A telltale was introduced into each branch of the inlet tube just outside the bell-jar. The one in the air line was a small gas-washing bottle containing a weak solution of $Ba(OH)_2$

colored with phenolphthalein. All air entering the jar bubbled through this solution and this telltale gave assurance that CO_2 had been effectively removed from the entering air. The rate of air flow, which was about 800 cc. per min., was regulated by adjusting the water pressure in a filter pump used to draw air through the apparatus. The telltale in the ethylene-supply line consisted of a gas-washing bottle of water, through which the rate of gas flow (0.8 cc. per min.) was indicated by the number of bubbles passing in a given time. The two jars of a test were in the same water reservoir and the temperature was regulated from time to time by the addition of warm or cool water as might be required.

When an experiment was begun, three similar, nearly mature celery plants with roots removed were weighed and placed in each of the two bell-jars, with their bases in a small amount of water. An air stream without any ethylene was passed through each jar until repeated measurements showed that the ratio of the two rates of CO_2 production had become fairly constant. Then ethylene was admitted to the air stream for one jar, at such a rate that the volumetric proportion of ethylene and air passing through the jar was approximately 1 to 1000. The other jar continued to receive air only. Successive measurements were then made on the rates of CO_2 production by the treated and untreated plants, at intervals of a few hours at first and later at intervals as long as 12 hr. Each experiment was continued until blanching was well advanced in the untreated as well as in the treated plants.

Five experiments of this sort were performed, with temperature about $19° \pm 3°$. The length of the experimental period was over 100 hr. in every instance, generally from 100 to 150 hr., with observation intervals about 5 or 6 hr. long. Mean hourly rates of CO_2 production for the successive observation intervals were computed as for 100 gm. of plant material. The rate in the control chamber (without ethylene) fluctuated irregularly, between about 4 or 5 mg. and about 9 or 10 mg. per hour. From 40 to 70 hr. after the introduction of ethylene into the continuous gas stream for the experiment jar, the rate of CO_2 production in that jar began to exceed the corresponding rate in the control jar, in experiments 1, 2, 3, and 5 of this series. The acceleration became gradually greater during an additional 80–

120 hr., after which it gradually decreased. At the end of the experiment, in these instances, the mean hourly rate of CO_2 production was about the same for both jars. The treated plants showed irregular fluctuations in the rate of CO_2 production throughout the whole period, similar to those noted for the untreated ones; but a marked acceleration apparently due to ethylene was clearly shown in all four of these tests. These fluctuations were not related to the temperature fluctuations nor were they related to night and day periods. For the observation intervals in which the accelerating influence of ethylene was most pronounced, the rate with ethylene was generally 10–30% more rapid than the corresponding control rate.

Experiment 4 showed no ethylene acceleration of CO_2 production in a period of 125 hr.; indeed there was some evidence of a slight but approximately maintained ethylene retardation of this process throughout the whole period.

It appeared from these results that this ethylene treatment might be found: (a) to exert no effect on CO_2 production if the period of treatment were shorter than 2 or 3 days or longer than 5 or 6 days; (b) to accelerate CO_2 production if the length of period were between about 3 and about 6 days, acceleration increasing to a maximum value and then decreasing to zero; (c) to retard CO_2 production slightly, but continuously, almost from the beginning of the test. The single wholly discrepant result and the apparently pronounced influence of period length on the results from each of the four experiments that were in general agreement indicated that such influential conditions need to be understood more precisely than they were in these tests. The plants were more nearly mature for the later experiments of this series than for the earlier ones, but it is remarkable that the fourth, and not the last test, was the discrepant one.

In the four experiments that gave similar results, blanching of the leaves was first evident at about the same time in both jars, but it progressed somewhat more rapidly in the jar continuously supplied with ethylene. In experiment 4, however (which was discrepant with respect to ethylene effect on CO_2 production), blanching was more rapid in the untreated plants. The time required for blanching, or for a given stage of blanching, was shorter for the later experiments than for the earlier ones, presumably on account of differences in the

degree of maturity of the plants used. In both the treated and the untreated plants the outer (older) leaves became blanched most rapidly and the effect of ethylene to hasten blanching was more pronounced for these leaves than for the inner (younger) ones.

It was observed in several cases that the inner (younger) petioles of the plants that had been exposed to ethylene elongated less than those of the plants exposed to air only, but no quantitative study of growth was made.

Measurements of catalase activity were made in experiment 5 of this series, by the method of leaf samples already referred to. In all cases catalase activity apparently decreased during the 4-day period but, as in the other similar tests of catalase activity, ethylene treatment showed no influence on either the rapidity or the extent of this change.

PRELIMINARY EXPERIMENTS WITH WHEAT SEEDLINGS.—From the experiments thus far considered no definite nor consistent conclusions might be drawn with regard to the influence of ethylene. Satisfactory repetition of these experiments was impossible, because one could not be sure of close similarity between two lots of plant material used at different times, since as the season advances the physiological state of the plants must become more or less altered. While these experiments were in progress it became increasingly evident that a clear picture of the nature of any influence exerted by ethylene on plant processes is hardly to be expected from experimentation unless the plant material used, as well as the methods employed, may be specified with sufficient precision to permit subsequent repetition of the experiments, either by the original experimenter or by others. Only under such circumstances may the results of several experiments be compared in a satisfactory manner. Nor may the results of several presumably like experiments performed at different times be combined, as by averaging, unless all effective variables are known to be practically alike for all. The plant material used should have as nearly as possible the same internal characteristics at the beginning for all presumably comparable experiments, and the experimental conditions should be adequately specified in all cases and nearly alike for all experiments whose results are to be averaged. Furthermore, the material used needs to be described

sufficiently well so that the same or another experimenter may be able at a later time to ascertain whether his material is in physiological capacity practically like that used in earlier experiments, or, if it differs considerably, just how it differs. These requirements, which are the same as for any other line of physiological experimentation, are of course difficult to meet, and the best that may be done is to plan each study so as to approach meeting them as nearly as possible.

It was suggested that wheat seedlings of similar genetic constitution, grown from pure-line seed that had not deteriorated significantly since being harvested, might be as suitable as any other equally available higher-plant material. If all the seeds of the stock used were essentially alike, if they did not become physiologically altered in storage during the progress of the experimentation, and if they were allowed to germinate and the resulting seedlings brought to a specified stage of development under specified standard conditions, then all the seedlings thus secured should be nearly alike in physiological capacity. Such variation as occurred among these "standard" seedlings might be cared for by the employment of a sufficiently large number of seedlings in each test, so that the several lots used in simultaneous or successive tests would be much more nearly alike statistically than the individual seedlings in any lot. To test these possibilities some preliminary experiments with wheat seedlings were performed at State College, in the summer of 1928. Nittany wheat, a pure-line variety bred by Dr. C. F. NOLL, of the Pennsylvania Agricultural Experiment Station, was used, of the crop of 1927.

In the first of these experiments 200 seeds were submerged in 450 ml. of SHIVE's (**48**) R_5C_2 nutrient solution in each of two wide-mouthed pint glass jars. Each liter of solution contained 0.1228 gm. of $Ca(NO_3)_2$, 0.2452 gm. of KH_2PO_4, and 0.3698 gm. of $MgSO_4$, and the osmotic value of the solution was approximately 0.175 atm. at 20°. Each culture jar was at once inclosed in one of the bell-jar chambers used in the celery experiments just described. The inlet tube of the chamber was extended so that it led to the bottom of the nutrient solution in the culture jar but the inner end of the outlet tube remained as before. The continuous current of gas was thus caused to bubble through the nutrient solution. A mixture of one volume of ethylene in 1000 volumes of air was passed through one culture from the be-

ginning, while air only was similarly passed through the other culture. The experiment was continued for 5 days, with a maintained temperature about 25°. The rate of CO_2 production in each culture was measured at intervals of about 12 hr., by absorption and titration, and the average amounts of elongation of shoots and of roots were ascertained for the 5-day period.

Carbon-dioxide production in the culture with ethylene was somewhat less rapid at first than that in the control culture; in the interval from the 20th to the 35th hour of the experiment the two rates were practically alike; and then the ethylene-treated seedlings were again less active than the others. Consequently it appears that the influence of the maintained ethylene pressure (0.1% in air) was generally to retard CO_2 production, but that this influence was somehow overcome for the first half of the second day of the experiment. This exceptional interval occurred before the seed coats were ruptured, in the latter part of the phase of swelling of the seeds. Of course the rate of CO_2 production was very low at first in both cultures and it increased with time, but its acceleration was less, on the whole, in the presence of ethylene than in its absence. For the entire period of 5 days, elongation of both shoots and roots was markedly retarded by the ethylene treatment. At the end of the experiment the average lengths of shoots and of roots were as follows:

	AVERAGE SHOOT LENGTH	AVERAGE ROOT LENGTH
Control culture.....	10.8 mm.	39.2 mm.
Culture with ethylene.....	3.2	10.2

The second of these experiments with wheat seedlings began with seedlings instead of dry seeds. These had been grown in indoor daylight, at a temperature of about 25°, from seeds on mosquito netting stretched across the top of an earthenware crock full of tap water. The shoots were about 60 mm. long, and the first leaf was partly extended and showed green color. There were 50 seedlings in each of two cultures, selected by pairs that were matched in size and general appearance, so that the two lots were of very nearly the same composition, so far as might be judged by appearance. These were treated as in the preceding experiment, except that the total concentration of the nutrient solution used in this instance was 10 times as

great as that of the solution used in the first. The rates of CO_2 production for untreated and ethylene-treated seedlings were nearly alike throughout the 5-day period. The rate of shoot elongation in the treated culture was lower than in the other during the first 2 days, for which period the average increments were 43.2 mm. (treated seedlings) and 54.1 mm. (untreated seedlings). After the first 2 days the rates of shoot elongation were nearly alike in both cultures but the shoots of the treated seedlings showed more bending than the others. At the end of 4 days the untreated seedlings were somewhat greener and more uniform in size than were the treated seedlings.

The third and fourth experiments in this preliminary series were similar to the first, but each culture began with 100 dry seeds, selected for uniformity in size and appearance. Temperature fluctuated irregularly between 25° and 26°, and the length of the whole experimental period was 4 days. The ethylene concentration used was the same as before, 0.1% in air by volume. Measurements of CO_2 produced with and without ethylene in the gas stream were made at intervals of about 12 hr. Ethylene retarded CO_2 production excepting for the initial 2-day period of swelling and bursting of the seeds, in which period there was no considerable difference in CO_2 production between the treated culture and the untreated one. The retardation in CO_2 rate became progressively more pronounced throughout the remaining 2 days of the period, and finally amounted to about 25%. No growth measurements were made in these two experiments but it was evident that shoot elongation was also retarded by ethylene.

Measurements of catalase and oxidase activity were made at the end of the fourth experiment, by means of 5-seedling samples. Three samples were taken from each of the six cultures. Sample no. 1b consisted of the five seedlings with longest shoots in the culture with ethylene, and the corresponding sample from the control culture (no. 1a) consisted of five seedlings selected for size to match those in no. 1b. The second sample from the ethylene-treated culture (no. 2b) consisted of somewhat smaller seedlings and these were matched as nearly as possible in the second sample from the untreated culture (no. 2a). The third samples (nos. 3b and 3a) were chosen to represent the average of the seedling population in each case. The seed-

lings of each sample were first freed of superficial moisture by light pressing between absorbent paper, after which the weight of the sample was ascertained. The seedlings of each sample (including the remaining endosperm) were triturated with their weight of powdered $CaCO_3$ and 50 times their weight of distilled water, and the preparation was then allowed to stand for 24 hr. at a temperature of 3°–4°.

Relative catalase activity was estimated for each preparation by the method used in the catalase experiments with celery. Relative oxidase activity was measured with a simplified Bunzel's apparatus, as described by HARVEY (**21**). After thorough stirring of the prepared sample, 5 ml. of the mixture were placed in one arm of the Y-shaped reaction tube and 1 ml. of a solution containing 1 gm. of pyrogallol in 100 ml. of water was introduced into the other arm. After the tube had been closed the two solutions were mixed and agitated continuously by means of a mechanical shaker making about 120 thrusts a minute, the manometer being read at 15-min. intervals for 1 hr. Oxidase activity was indicated by reduction of gas pressure inside the reaction tube, due to oxygen absorption by the pyrogallol under the influence of the oxidizing enzyms. All six tests were made simultaneously. The temperature of the tube and its contents was 27.5°.

The results of these measurements on catalase and oxidase are presented in table I. The index of relative catalase activity is of course the reciprocal of the time required for the release of a unit of oxygen, and larger numbers of seconds consequently represent lower degrees of catalase activity. On the other hand, the pressure decrements given are themselves taken as indices of oxidase activity. Throughout the table the values from ethylene-treated seedlings are in bold-face type.

The first pair of samples (1a, 1b) showed greater catalase activity and less oxidase activity for the ethylene-treated seedlings than for the untreated ones, but the converse is true for the remaining two pairs of samples. It is clear that there was much variability among the control seedlings with respect to these estimates of enzym activity, and it seems that the influence of ethylene probably differed according to the vigor of the seedlings. Ethylene apparently *increased* catalase activity and *decreased* oxidase activity in the more vigorous

seedlings but it appears to have *decreased* catalase activity and *increased* oxidase activity in somewhat less vigorous seedlings. This remarkable discrepancy suggests that serious difficulties of variability might need to be overcome if a consistent study of these physiological activities were to be undertaken by means of experiments of this sort. About all that may be said in this particular connection is that ethylene might be expected either to accelerate or to retard

TABLE I

CATALASE AND OXIDASE ACTIVITY OF WHEAT SEEDLINGS 4 DAYS AFTER DRY SEEDS HAD BEEN PLACED UNDER NUTRIENT SOLUTION THROUGH WHICH AIR OR ETHYLENE-AIR MIXTURE (1:1000) BUBBLED CONTINUALLY; TEMPERATURE ABOUT 25°

Sample no.	Treatment	Weight of sample (5 seedlings) (mg.)	Catalase activity; time (in seconds) required to release		Oxidase activity; manometric pressure reduction after consecutive 15-min. intervals, in mm. of Hg			
			1 ml. of oxygen	2 ml. of oxygen	1st interval	2nd interval	3rd interval	4th interval
1a....	Control (air only)	512	82	433	4.5	14.0	23.0	31.0
1b....	Air and ethylene	485	**55**	**230**	**3.5**	**12.4**	**20.8**	**29.5**
2a....	Control (air only)	442	128	880	4.1	12.2	20.0	26.4
2b....	Air and ethylene	464	**384**	**1590**	**7.1**	**16.0**	**23.0**	**30.2**
3a....	Control (air only)	526	35	75	2.5	10.0	17.6	23.5
3b....	Air and ethylene	452	**265**	**1345**	**5.4**	**15.5**	**27.5**	**33.3**

these enzymatic processes, or to exert no influence upon them, and that if one of the processes were accelerated the other would presumably be retarded.

Main series of experiments with wheat seedlings

Experimental procedure

The results of the preliminary experiments already described strengthened our conviction that ethylene influence on plant processes may vary in direction as well as in amount, not only with reference to ethylene treatment and according to the kind of plant material considered, its current developmental phase and physiological

vigor, but also with reference to the prevailing complex of non-ethylene conditions. Since wheat seedlings appeared to be promising material for a study of some kinds of ethylene effects as influenced by conditions other than those dependent on ethylene itself, a consistently arranged series of experiments with wheat seedlings was planned and carried out, at the Laboratory of Plant Physiology of the Johns Hopkins University, in 1928–29. An attempt was made to take into account in a quantitative way some of the most readily suggested conditions that might influence the direction and extent of ethylene effects. The partial pressures of oxygen, of carbon dioxide and other volatile metabolic products, and of ethylene were all approximately maintained in the seedling environment for each experiment, through the employment of continuously flowing gas mixtures without CO_2 and with known contents of oxygen, nitrogen, and ethylene. A greatly superfluous supply of moisture to the seedlings was maintained by having the latter submerged in a relatively large volume of a standard nutrient solution, which also gave assurance that the supplies of mineral nutrients were initially alike for all experiments and did not alter very rapidly during the relatively short experimental period. Temperature was maintained for each experiment and light was excluded. A standard procedure was developed, so that experiments might be repeated and the results of any experiment might be comparable with those of similar or partially similar experiments performed at other times. Temperature, time, the partial pressure of oxygen and the presence or absence of ethylene at a single specified partial pressure were the experimental variables, and their effects were studied throughout an extensive series of combinations.

The experiments without ethylene were all performed first, constituting in themselves a consistent study of the influence of oxygen pressure, temperature, and time on CO_2 production and shoot elongation under the standard background conditions. The main results of that experiment series have already been reported by MACK (**32**). The experiments with ethylene were performed after those without ethylene had been completed, and the earlier series are of course the respective controls for the later series. The experimental technique employed was the same for both series, excepting such modifications

as were necessitated by the introduction of ethylene into the environmental complex or by limitations of time for the study as a whole. This technique and our computation procedures have been mostly described in detail by MACK (**32**), to whose paper reference should be made in connection with critical study of the present report.

Five different maintained temperatures (10°, 15°, 20°, 25°, 30°) were employed in each series. There were twelve different gas mixtures without ethylene, consisting of nitrogen and oxygen, with oxygen contents of 0.6, 3.1, 6.3, 9.8, 16.0, 20.0, 30.0, 50.0, 75.0, 90.0, 95.0, and 98.3% by volume. For the experiments with ethylene the nitrogen-oxygen mixtures containing 3.1, 90.0, 95.0, and 98.3% of oxygen were omitted and percentages of 16.6 and 21.0 were employed instead of 16.0 and 20.0. These last differences will be ignored in the following presentation and it will be understood that 16 and 20% mean 16.6 and 21% when reference is had to the ethylene series. To each of these eight nitrogen-oxygen mixtures ethylene was added to make up 0.1% of the total gas volume. In every experiment the gas mixture bubbled continuously through the nutrient solution in which the seedlings were submerged, renewing the oxygen supply and removing CO_2 and any other volatile products of metabolism that may have been eliminated. The total gas pressure above the culture solution was very little above that of the ordinary atmosphere, fluctuating slightly with weather changes. The rate of gas flow was about 20 ml. per minute, which had been shown, in special tests, to be more than sufficient for maximal CO_2 production and growth, so far as oxygen supply was concerned, for all the temperature-oxygen combinations tested without ethylene. The CO_2 brought away by the gas stream was collected in $Ba(OH)_2$ solution and measured by titration at the same specified intervals in all experiments of both series; namely, 4, 10, 22, 34, and 46 hr. after the end of a preliminary 2-hr. interval for transfer and adjustment. The five observation intervals for measurement of CO_2 were therefore 4, 6, 12, 12, and 12 hr. long, respectively, and there were 300 different combinations of time, temperature, and partial oxygen pressure without ethylene, and 200 different combinations of the same variables with ethylene.

The standard culture solution used for all experiments in both se-

ries was SHIVE's solution R_5C_2 (**48**), calculated to have an osmotic value of 1.75 atm. at 20°. Each liter contained 0.1228 gm. of $Ca(NO_3)_2$, 0.2452 gm. of KH_2PO_4, and 0.3698 gm. of $MgSO_4$; no iron was added. The distilled water used was from a Barnstead still. All cultures were in 300-ml. Erlenmeyer flasks of Pyrex chemically-resistant glass, each with 250 ml. of solution and 100 standard seedlings. The standard solution must have contained very small amounts of other substances than are here mentioned, owing to inevitable impurities in the water and salts used and slight solubility of the glass containers. Some changes doubtless occurred in the essential constitution of the solution during the 48-hr. experimental period, notably because of absorption and excretion by the seedlings, but it may be tentatively supposed that such changes did not differ enough from experiment to experiment to produce significant difference in the results. Of course our experimental technique would have approached logical perfection more nearly if the culture solutions had been continuously renewed, which is highly desirable in all solution-culture experiments, as has been emphasized by TRELEASE and LIVINGSTON (**55**), SHIVE and STAHL (**49**), and PIRSCHLE (**36**). To develop methods and procedures for continuous solution flow that might be applied while the other requirements of these experiments were satisfied, however, would have required more time than was at our disposal. In several special tests, solutions that had previously been used as in the regular experiments, but for only 22 hr., gave somewhat more rapid CO_2 production in the first 6 hr. than was given by corresponding fresh solutions, but that difference was not evident in subsequent intervals. The question thus raised is worthy of special study.

Five cultures with different temperatures but all with gas mixture from the same tank were carried out at one time. The flasks were prepared beforehand, being charged with nutrient solution and allowed to attain the desired temperature in their respective temperature chambers before the introduction of the seedlings.

The seedlings used were all grown from the same stock of Nittany wheat as had been used for the preliminary experiments with wheat seedlings. For each set of five cultures, 1000 selected seeds were introduced into 500 ml. of distilled water in a 600-ml. flask. These

were allowed to swell and germinate for a germination period of 42 hr. at a temperature of 19.5°, during which time air was bubbled through the liquid at a rate of about 30 liters per hour, rapidly enough to produce a vigorous stirring of the water and some movement of the seeds. Nearly all of the seeds germinated with this treatment. (For a study of the influence of rate of air flow in such cultures, see TANG'S (**54**) recent paper.) At the end of the germination period, when coleoptiles had burst forth and roots were about to emerge from the coleorhizas, 100 selected seedlings were transferred to nutrient solution in each of the five prepared culture flasks, which were immediately returned to their respective temperature chambers; the flow of the requisite gas mixture was then started. The first interval for measuring CO_2 production began 44 hr. after the dry seeds had been placed in water for germination.

No attempt was made to avoid possible development of microorganisms, either in the germination cultures or in the regular experiments. No fungus was observed, but there was doubtless considerable development of bacteria, and perhaps of other microorganisms, on the seeds and seedlings and in the culture medium, for, as has been said, the same charge of liquid was used throughout the experimental period without renewal. If the influence of microorganisms on the rate of CO_2 production or on the rate of shoot elongation was significant, it is embraced in the general background complex for all these experiments. But the possibility of such influence should naturally be borne in mind in connection with the interpretation of the results of such studies as this. It should be explained, perhaps, that this study deals not just with the activities of wheat seedlings as living material but with their activities along with the concomitant activities of any and all microorganisms that may have been present in the cultures. Our discussion proceeds, however, as though microorganisms were known to have been uninfluential with respect to the process rates considered.

The experiments with ethylene differed essentially from their respective controls (the corresponding ones without ethylene) only in that their flowing gas mixtures all contained 0.1% of ethylene by volume. Ethylene was measured into the mixing cylinder before either oxygen or nitrogen was introduced, so that its pressure could

be measured with a mercury manometer instead of with the less accurate pressure gauge used for the much greater pressures of oxygen and nitrogen. The ethylene used was of the grade commonly employed for anaesthesia, and was obtained from the Kansas City Oxygen Gas Company. Ethylene was regularly admitted into the mixing cylinder until the pressure therein was 1 pound (5.1 cm. on the manometer scale); oxygen and nitrogen were then admitted in the requisite proportions, making up a total pressure of 1000 pounds, read on the pressure gauge. The oxygen percentage of each gas mixture was ascertained, for a sample, by absorption of oxygen with phosphorus (as was also done in the case of the controls), and this percentage was corrected, if necessary, by adding small amounts of oxygen or nitrogen until sample tests showed that the desired percentage had been very nearly attained.

Only one ethylene concentration was tested. This concentration (0.1% by volume) is the same as was used in the preliminary experiments, described in the earlier part of this paper. As has been noted, it has been recommended for blanching celery, ripening tomatoes, etc. It has been reported, for example, by WALLACE (**59**) and DENNY (**7**), that the nature of the effects of ethylene was qualitatively the same for a wide range of concentrations, although it might differ quantitatively for relatively small differences in concentration. It is obvious, however, that much further experimentation will be required when the concentration of ethylene comes to be studied as an experimental variable in this sort of investigation.

At the end of the fifth interval of each experiment (48 hr. after the standard seedlings had been placed in the experimental environment), the average shoot length of the longest ten seedlings in each culture was ascertained and this average was taken to represent the growth activity of the plantlets for the whole experimental period.

NUMERICAL DATA

The primary results of this study comprise (1) the mean hourly rates of CO_2 production by 100 seedlings for each of the five observation intervals, and (2) the growth indices secured at the end of the experimental period. These values were simply averaged for each group of two or more like experiments and the resulting final averages are shown in tables II–VI. The values from the separate experi-

TABLE II

AVERAGE HOURLY RATES OF CO_2 PRODUCTION IN 1ST AND 2ND INTERVALS, WITH CORRESPONDING ETHYLENE EFFECTS

Oxygen pressure (%)	Temperature (° C.)	First interval (3rd to 6th hr. inclusive)			Second interval (7th to 12th hr. inclusive)		
		Without ethylene (mg.)	With ethylene, 0.1% (mg.)	Ethylene effect (%)	Without ethylene (mg.)	With ethylene, 0.1% (mg.)	Ethylene effect (%)
0.6	10	0.36	0.34	− 6	0.26	0.25	− 4
	15	0.46	0.46	0	0.41	0.39	− 5
	20	0.63	0.63	0	0.61	0.61	0
	25	0.74	0.81	+10	0.79	0.78	− 1
	30	0.96	0.97	+ 1	1.11	0.96	−13
6.3	10	0.38	0.38	0	0.31	0.31	0
	15	0.61	0.58	− 5	0.56	0.59	+ 5
	20	0.73	0.67	− 8	0.77	0.76	− 1
	25	0.93	0.89	− 4	1.14	1.08	− 5
	30	1.16	1.23	+ 6	1.72	1.73	+ 1
9.8	10	0.42	0.42	0	0.34	0.37	+ 9
	15	0.56	0.63	+12	0.54	0.62	+15
	20	0.86	0.81	− 6	0.80	0.88	+10
	25	1.02	1.03	+ 1	1.37	1.38	+ 1
	30	1.21	1.32	+ 9	1.86	1.99	+ 7
16.0	10	0.44	0.41	− 7	0.41	0.41	0
	15	0.58	0.60	+ 3	0.56	0.55	− 2
	20	0.76	0.80	+ 5	0.83	0.92	+11
	25	0.97	1.12	+16	1.20	1.44	+20
	30	1.23	1.32	+ 7	1.75	1.97	+13
20.0	10	0.39	0.39	0	0.41	0.40	− 2
	15	0.63	0.59	− 8	0.64	0.65	+ 2
	20	0.82	0.80	− 2	0.84	0.91	+ 8
	25	0.95	0.94	− 1	1.16	1.21	+ 4
	30	1.30	1.35	+ 4	1.76	1.75	− 1
30.0	10	0.44	0.50	+14	0.54	0.56	+ 4
	15	0.71	0.72	+ 1	0.72	0.73	+ 1
	20	0.83	0.95	+14	0.94	1.13	+20
	25	1.05	1.12	+ 7	1.24	1.46	+18
	30	1.34	1.45	+ 8	1.69	1.92	+14
50.0	10	0.45	0.51	+13	0.56	0.54	− 4
	15	0.74	0.72	− 3	0.85	0.83	− 2
	20	0.94	0.90	− 4	1.05	1.07	+ 2
	25	1.20	1.20	0	1.44	1.52	+ 6
	30	1.60	1.56	− 3	2.02	1.90	− 6
75.0	10	0.47	0.56	+19	0.59	0.58	− 2
	15	0.82	0.77	− 6	0.98	1.01	+ 3
	20	1.10	0.96	−13	1.37	1.31	− 4
	25	1.38	1.38	0	1.81	1.95	+ 8
	30	1.91	1.90	− 1	2.56	2.66	+ 4

ments also are given, excepting those for CO_2 production in the 1st and 2nd intervals (table II), for it seems desirable to show the kind and extent of the deviation or variability encountered; they are omitted for CO_2 production in the first two intervals to conserve space and because our discussion of CO_2 production will deal mainly with the data for the last three intervals. As was suggested by MACK (**32**), the 2-hr. interval for adjustment (at the beginning of each experiment) may perhaps have been somewhat too short a period; there may have been some after-effects of previous culture or of transfer still more or less prevalent after the beginning of the first observation interval. The seedlings were of course larger and more active in the later intervals and the actual amounts of CO_2 measured were correspondingly larger, with relatively smaller errors of titration. Furthermore, the last three intervals were of the same length (12 hr.), while the 1st and 2nd intervals were shorter and of unequal length (4 and 6 hr. respectively), and the CO_2 rates for the last three intervals are somewhat more clearly consistent in their own group and in the whole series of five than are the corresponding data for the first two intervals. Some of the values given in these tables have previously been published by MACK (**32**).

Only the final averages will be considered. Each is derived from two or more like tests, as is seen from tables III–VI, but the number of duplicates is generally far too small to warrant any attempt to apply statistical methods, even if that might be deemed desirable in studies of this sort. The degree of significance of the final averages is to be appraised in terms of their consistency, as will appear from the following discussions.

Because the CO_2 data for the last three intervals are remarkably consistent, they were combined to give a single series to represent those three intervals taken together, and the resulting average CO_2 rates are set forth in table VII. They will be used in comparing CO_2 production with shoot elongation. As has been said, the indices of shoot elongation are all based on measurements made at the end of the whole experimental period, and they really represent the germination and experimental periods combined. However, the shoots of the standard seedlings were never more than 1 mm. long when brought under the experimental conditions.

Each of the final average values for CO_2 production without ethylene (control values) was subtracted from the corresponding value for ethylene treatment and the difference divided by the control

TABLE III

DETAILED DATA ON CO_2 PRODUCTION IN 3RD INTERVAL (13TH TO 24TH HR. INCLUSIVE)

Oxygen pressure (%)	Temperature (°C.)	Hourly rates of CO_2 production (mg.)				Ethylene effect (%)
		Without ethylene		With ethylene, 0.1 %		
		Separate tests	Ave.	Separate tests	Ave.	
0.6	10	0.28, 0.27, 0.27	0.27	0.26, 0.25	0.26	− 4
	15	0.45, 0.43, 0.50	0.46	0.46, 0.48	0.47	+ 2
	20	0.66, 0.64, 0.68	0.66	0.67, 0.67	0.67	+ 2
	25	1.01, 0.95, 0.83	0.93	0.88, 0.94	0.91	− 2
	30	1.43, 1.57, 1.11	1.37	1.25, 1.35	1.30	− 5
6.3	10	0.36, 0.32	0.34	0.33, 0.31	0.32	− 6
	15	0.57, 0.64	0.61	0.71, 0.71	0.71	+16
	20	1.08, 1.13	1.11	0.91, 1.18	1.05	− 5
	25	1.93, 1.68	1.81	1.67, 1.73	1.70	− 6
	30	2.55, 2.02	2.29	2.13, 2.36	2.25	− 2
9.8	10	0.34, 0.32	0.33	0.39, 0.36, 0.29	0.35	+ 6
	15	0.51, 0.52	0.52	0.70, 0.60, 0.78	0.69	+33
	20	1.07, 1.05	1.06	1.30, 1.10, 1.27	1.22	+15
	25	1.88, 2.02	1.95	2.16, 2.40, 2.02	2.19	+12
	30	2.37, 2.34	2.36	2.62, 2.96, 2.80	2.79	+18
16.0	10	0.42, 0.34, 0.43, 0.49	0.44	0.48, 0.46, 0.48	0.47	+ 7
	15	0.62, 0.63, 0.59, 0.63	0.62	0.67, 0.76, 0.72	0.72	+16
	20	1.11, 1.07, 0.97, 1.18	1.08	1.30, 1.17, 1.15	1.21	+12
	25	1.89, 1.47, 1.71, 1.88	1.74	2.01, 1.84, 1.85	1.90	+ 9
	30	2.62, 2.54, 2.70	2.62	2.29, 2.75, 2.66	2.57	− 2
20.0	10	0.36, 0.37, 0.52, 0.51, 0.45, 0.48, 0.47	0.45	0.44, 0.38	0.41	− 9
	15	0.77, 0.76, 0.83, 0.84, 0.73, 0.71, 0.73	0.77	0.77, 0.82	0.80	+ 4
	20	1.07, 1.03, 1.09, 1.06, 1.02, 1.04, 1.16	1.07	1.14, 1.12	1.13	+ 6
	25	1.37, 1.35, 1.59, 1.42, 1.61, 1.40, 1.47	1.46	1.33, 1.46	1.40	− 4
	30	2.13, 2.38, 2.47, 2.37, 2.32, 2.14, 2.14	2.28	2.17, 2.13	2.15	− 6
30.0	10	0.58, 0.59, 0.60	0.59	0.66, 0.55	0.61	+ 3
	15	0.87, 0.80, 0.83	0.83	0.89, 0.92	0.91	+10
	20	1.10, 1.25, 1.27	1.21	1.32, 1.34	1.33	+10
	25	1.61, 1.73, 1.66	1.67	1.78, 1.78	1.78	+ 7
	30	2.07, 2.41, 2.28	2.25	2.43, 2.48	2.46	+ 9
50.0	10	0.67, 0.71	0.69	0.63, 0.58	0.61	−12
	15	0.96, 0.98	0.97	1.03, 0.93	0.98	+ 1
	20	1.31, 1.54	1.43	1.38, 1.31	1.35	− 6
	25	1.79, 2.07	1.93	2.02, 1.94	1.98	+ 3
	30	2.94, 2.82	2.88	2.46, 2.68	2.57	−11
75.0	10	0.77, 0.78, 0.73, 0.73	0.75	0.75, 0.66	0.71	− 5
	15	1.16, 1.37, 1.25, 1.42	1.30	1.33, 1.13	1.23	− 5
	20	2.05, 1.87, 1.92, 2.08	1.98	2.04, 1.62	1.83	− 8
	25	2.65, 2.78, 2.70, 3.05	2.80	3.19, 2.50	2.85	+ 2
	30	3.92, 3.68, 4.04	3.88	3.96, 3.02	3.79	− 2

value. The quotient was finally multiplied by 100, to give an index of ethylene effect expressed as a percentage based on the corresponding control value. This index may be: (1) positive (when the aver-

age rate is greater with ethylene than without it; that is, when ethylene apparently accelerated CO_2 production); (2) zero (when ethylene treatment showed no effect); or (3) negative (when the

TABLE IV

DETAILED DATA ON CO_2 PRODUCTION IN 4TH INTERVAL (25TH TO 36TH HR. INCLUSIVE)

Oxygen pressure (%)	Temperature (° C.)	Hourly rates of CO_2 production (mg.)				Ethylene effect (%)
		Without ethylene		With ethylene, 0.1 %		
		Separate tests	Ave.	Separate tests	Ave.	
0.6	10	0.29, 0.27, 0.27	0.28	0.32, 0.28	0.30	+ 7
	15	0.47, 0.45, 0.50	0.47	0.51, 0.49	0.50	+ 6
	20	0.67, 0.71, 0.72	0.70	0.73, 0.66	0.70	0
	25	1.07, 1.05, 0.96	1.03	1.06, 1.10	1.08	+ 5
	30	1.59, 1.43, 1.24	1.42	1.37, 1.55	1.46	+ 3
6.3	10	0.36, 0.38	0.37	0.43, 0.31	0.37	0
	15	0.75, 0.89	0.82	0.97, 0.89	0.93	+13
	20	1.42, 1.33	1.38	1.40, 1.45	1.43	+ 4
	25	2.28, 1.83	2.06	2.09, 1.82	1.96	− 5
	30	2.84, 2.28	2.56	2.90, 2.72	2.81	+10
9.8	10	0.34, 0.38	0.36	0.51, 0.44	0.48	+33
	15	0.67, 0.73	0.70	0.98, 0.77	0.88	+26
	20	1.19, 1.16	1.18	1.75, 1.36	1.56	+32
	25	2.38, 2.23	2.31	2.50, 2.58	2.54	+10
	30	3.23, 3.15	3.19	3.23, 3.40	3.32	+ 4
16.0	10	0.47, 0.51, 0.53, 0.53	0.51	0.55, 0.53, 0.55	0.54	+ 6
	15	0.68, 0.75, 0.84, 0.81	0.77	0.91, 0.93, 0.86	0.90	+17
	20	1.18, 1.24, 1.04, 1.22	1.17	1.50, 1.36, 1.22	1.36	+16
	25	2.20, 2.17, 2.15, 2.30	2.21	2.46, 2.35, 2.40	2.40	+ 9
	30	3.87, 3.70, 3.70	3.76	3.06, 3.73, 4.06	3.62	− 4
20.0	10	0.39, 0.47, 0.66, 0.58, 0.54, 0.54	0.53	0.53, 0.40	0.47	−11
	15	0.94, 0.93, 1.01, 0.98, 0.91, 0.88	0.94	0.91, 0.92	0.92	− 2
	20	1.18, 1.29, 1.31, 1.41, 1.29, 1.50	1.33	1.32, 1.18	1.25	− 6
	25	1.76, 1.75, 2.04, 2.07, 1.77, 1.98	1.90	1.60, 1.76	1.68	−12
	30	2.78, 3.27, 3.57, 3.49, 3.01, 3.21	3.22	3.06	3.06	− 5
30.0	10	0.68, 0.72, 0.71	0.70	0.75, 0.66	0.71	+ 1
	15	1.07, 1.02, 1.03	1.04	1.08, 1.14	1.11	+ 7
	20	1.31, 1.52, 1.54	1.46	1.72, 1.65	1.69	+16
	25	2.03, 2.07, 1.90	2.00	2.40, 2.27	2.34	+17
	30	2.84, 3.22, 3.27	3.11	3.60, 3.43	3.52	+13
50.0	10	0.82, 0.89	0.86	0.72, 0.73	0.73	−15
	15	1.26, 1.23	1.25	1.23, 1.22	1.23	− 2
	20	1.79, 1.97	1.88	1.76, 1.69	1.73	− 8
	25	2.62, 2.88	2.75	2.40, 2.66	2.53	− 8
	30	4.51, 4.75	4.63	3.78, 3.98	3.88	−16
75.0	10	0.87, 0.88, 0.77, 0.75	0.82	0.90, 0.73	0.82	0
	15	1.53, 1.78, 1.57, 1.75	1.66	1.79, 1.51	1.65	− 1
	20	2.86, 2.56, 2.82, 3.05	2.82	3.24, 2.47	2.86	+ 1
	25	3.77, 3.88, 3.94, 4.37	3.99	4.45	4.45	+12
	30	5.20, 5.00, 5.47	5.22	5.48, 4.80	5.14	− 2

presence of ethylene apparently acted to retard CO_2 production). Percentage indices of ethylene effect on shoot elongation were computed in a similar manner. In the columns so designated (tables II–

VII) these percentages of ethylene effect are shown for CO_2 production in each of the five observation intervals and in the last three intervals combined, as well as for shoot elongation.

TABLE V

DETAILED DATA ON CO_2 PRODUCTION IN 5TH INTERVAL (37TH TO 48TH HR. INCLUSIVE)

Oxygen pressure (%)	Temperature (° C.)	Hourly rates of CO_2 production (mg.)				Ethylene effect (%)
		Without ethylene		With ethylene, 0.1%		
		Separate tests	Ave.	Separate tests	Ave.	
0.6	10	0.31, 0.27, 0.33	0.30	0.32, 0.27	0.30	0
	15	0.52, 0.50, 0.49	0.50	0.50, 0.50	0.50	0
	20	0.76, 0.83, 0.80	0.80	0.79, 0.75	0.77	− 4
	25	1.32, 1.25, 1.12	1.23	1.13, 1.14	1.14	− 7
	30	1.59, 1.54, 1.30	1.48	1.48, 1.72	1.60	+ 8
6.3	10	0.42, 0.47	0.45	0.46, 0.46	0.46	+ 2
	15	1.00, 1.04	1.02	1.26, 1.20	1.23	+21
	20	1.50, 1.58	1.54	1.73, 1.61	1.67	+ 8
	25	2.30, 2.30	2.30	2.52, 2.22	2.37	+ 3
	30	3.36, 2.76	3.06	3.67, 3.58	3.63	+19
9.8	10	0.40, 0.38	0.39	0.53, 0.52	0.53	+36
	15	0.87	0.87	1.17, 1.07	1.12	+29
	20	1.53, 1.57	1.55	1.91, 1.89	1.90	+23
	25	3.09, 2.76	2.93	2.91, 2.98	2.95	+ 1
	30	3.82, 3.61	3.72	3.54, 4.16	3.85	+ 3
16.0	10	0.55, 0.56, 0.62, 0.65	0.60	0.62, 0.61	0.62	+ 3
	15	0.76, 0.87, 1.03, 0.99	0.91	1.08, 1.04, 1.04	1.05	+15
	20	1.42, 1.34, 1.42, 1.53	1.43	1.81, 1.73, 1.54	1.69	+18
	25	2.74, 2.75, 3.32, 3.07	2.97	3.28, 3.19, 2.92	3.13	+ 5
	30	5.03, 4.33, 4.63	4.66	3.89, 4.20, 4.55	4.21	−10
20.0	10	0.46, 0.49, 0.73, 0.68, 0.63, 0.68	0.61	0.56, 0.60	0.58	− 5
	15	1.12, 1.08, 1.19, 1.22, 1.22, 1.09	1.15	1.07, 1.22	1.15	0
	20	1.50, 1.69, 1.58, 1.69, 1.71, 1.87	1.67	1.63, 1.43	1.53	− 8
	25	2.26, 2.34, 2.72, 2.49, 2.33, 2.51	2.46	2.06, 2.58	2.32	− 6
	30	3.63, 3.82, 4.50, 4.03, 4.00	4.00	3.92, 4.52	4.22	+ 6
30.0	10	0.81, 0.84, 0.83	0.83	0.81, 0.81	0.81	− 2
	15	1.35, 1.33, 1.33	1.34	1.40, 1.38	1.39	+ 4
	20	1.71, 1.84, 1.95	1.83	2.33, 2.15	2.24	+22
	25	2.80, 2.77, 2.68	2.75	3.36, 3.05	3.21	+17
	30	4.10, 4.50, 4.43	4.34	4.68, 4.38	4.53	+ 4
50.0	10	1.02, 1.07	1.05	0.86, 0.88	0.87	−17
	15	1.66, 1.75	1.71	1.61, 1.50	1.56	− 9
	20	2.44, 2.72	2.58	2.20, 2.28	2.24	−13
	25	3.50, 4.24	3.87	3.33, 3.50	3.42	−12
	30	5.67, 5.95	5.81	4.61, 5.32	4.97	−14
75.0	10	1.08, 1.17, 0.97, 1.00	1.06	1.16, 0.86	1.01	− 5
	15	2.34, 2.41, 2.40, 2.50	2.41	2.42, 2.06	2.24	− 7
	20	3.81, 3.66, 3.95, 3.83	3.81	4.26, 3.70	3.98	+ 4
	25	4.42, 5.17, 4.83, 5.32	4.94	5.65, 5.04	5.35	+ 8
	30	6.06, 5.99, 6.36	6.14	5.66, 5.97	5.82	− 5

Those tests without ethylene for which there were no corresponding tests with ethylene are not generally considered in this paper and the data for them do not appear in the tables. They were with oxy-

gen pressures of 3.1, 90.0, 95.0, and 98.3%. Because of lack of time for further experimentation ethylene treatment with these oxygen pressures was not tested. The results of the tests without ethylene,

TABLE VI

DETAILED DATA ON SHOOT ELONGATION (WHOLE PERIOD)

Oxygen pressure (%)	Temperature (° C.)	Average lengths of longest 10 shoots (mm.)				Ethylene effect (%)
		Without ethylene		With ethylene, 0.1%		
		Separate tests	Ave.	Separate tests	Ave.	
6.3	10	1.7, 1.4	1.6	0, 0	0	−100
	15*	1.7, 1.4	1.6	0, 0	0	−100
9.8	10	2.4, 2.3	2.4	1.6, 1.6	1.6	− 33
	15	2.9, 2.7	2.8	1.8, 2.1	2.0	− 29
	20*	2.6, 2.6	2.6	0, 0	0	−100
16.0	10	2.6, 2.6, 3.2, 2.9	2.8	2.5, 2.7, 2.3	2.5	− 11
	15	3.5, 3.3, 4.0, 4.2	3.8	3.5, 3.6, 3.4	3.5	− 8
	20	3.0, 3.2, 2.5, 3.4	3.0	3.5, 3.9, 3.4	3.6	+ 20
	25*	2.5, 2.5, 2.3, 2.4	2.4	2.3, 2.2, 2.0	2.2	− 8
20.0	10	3.0, 2.7, 2.7, 3.6, 3.4, 2.7, 2.7	3.0	1.2, 2.2	2.0	− 33
	15	5.4, 4.1, 4.7, 6.3, 5.9, 3.8, 3.7	4.8	3.3, 3.5	3.4	− 29
	20	5.8, 7.1, 8.8, 7.0, 9.3, 6.4, 5.6	7.2	3.9, 4.4	4.2	− 42
	25	4.8, 8.2, 11.2, 5.4, 10.2, 6.0, 4.8	7.2	4.5, 4.9	4.7	− 35
	30	2.3, 2.2, 2.5, 2.0, 2.5, 1.9, 2.0	2.2	0, 0	0	−100
30.0	10	2.7, 3.4, 2.9	3.0	2.6, 2.5	2.6	− 13
	15	5.0, 4.7, 5.4	5.0	4.7, 3.7	4.2	− 16
	20	10.4, 6.9, 10.3	9.2	6.6, 5.3	5.9	− 36
	25	7.4, 5.7, 10.3	7.8	6.1, 5.1	5.6	− 28
	30	3.1, 3.1, 4.1	3.4	2.8, 3.0	2.9	− 15
50.0	10	3.0, 3.0	3.0	2.5, 2.4	2.5	− 17
	15	5.4, 5.4	5.4	3.9, 4.3	4.1	− 24
	20	10.2, 7.8	9.0	4.7, 6.0	5.4	− 40
	25	11.4, 10.6	11.0	5.6, 5.0	5.3	− 52
	30	2.8, 4.7	3.8	4.4, 4.2	4.3	+ 13
75.0	10	2.2, 2.4, 2.3, 2.5	2.4	2.2, 1.8	2.0	− 17
	15	3.0, 5.1, 4.0, 3.9	4.0	4.5, 4.4	4.5	+ 13
	20	6.1, 7.6, 7.6, 5.0	6.6	7.3, 7.9	7.6	+ 15
	25	7.8, 9.7, 8.0, 6.6	8.0	7.3, 7.5	7.4	− 8
	30	3.1, 6.9, 3.8, 3.8	4.4	5.9, 6.1	6.0	+ 36

* There was no measurable shoot growth, either with or without ethylene, for any tested temperature with oxygen pressure of 0.6%. The same is true for 20°, 25°, and 30° with oxygen pressure of 6.3%; for 25° and 30° with oxygen pressure of 9.8%; and for 30° with oxygen pressure of 16.0%. Consequently these combinations of temperature and oxygen pressure do not appear in this table.

however, are given by MACK (**32**), and some of them will be mentioned in our discussion.

PRIMARY GRAPHS OF CO_2 PRODUCTION

METHOD OF PLOTTING.—The final average hourly rates of CO_2 production in each of the last three observation intervals and in these three intervals taken together are shown graphically in figure 1.

TABLE VII

AVERAGE HOURLY RATES OF CO_2 PRODUCTION IN 3RD, 4TH, AND 5TH INTERVALS COMBINED (13TH TO 48TH HR.), TOGETHER WITH CORRESPONDING ETHYLENE EFFECTS ON CO_2 PRODUCTION AND ON SHOOT ELONGATION (GROWTH DATA ARE FROM TABLE VI)

Oxygen pressure (%)	Temperature (° C.)	Average hourly rate of CO_2 production (mg.)		Ethylene effect (%)	
		Without ethylene	With ethylene, 0.1%	On CO_2 production	On shoot elongation
0.6	10	0.28	0.29	+ 4	.....
	15	0.48	0.49	+ 2	.....
	20	0.72	0.71	− 2	.....
	25	1.06	1.04	− 2	.....
	30	1.42	1.45	+ 2	.....
6.3	10	0.39	0.38	− 3	−100
	15	0.82	0.96	+17	−100
	20	1.34	1.38	+ 3	.....
	25	2.06	2.01	− 2	.....
	30	2.64	2.90	+10	.....
9.8	10	0.36	0.45	+25	− 33
	15	0.70	0.90	+29	− 29
	20	1.26	1.56	+24	−100
	25	2.40	2.56	+ 7	.....
	30	3.09	3.32	+ 7	.....
16.0	10	0.52	0.54	+ 4	− 11
	15	0.77	0.89	+16	− 8
	20	1.23	1.42	+15	+ 20
	25	2.31	2.48	+ 7	− 8
	30	3.68	3.47	− 6	.....
20.0	10	0.53	0.49	− 8	− 33
	15	0.95	0.96	+ 1	− 29
	20	1.36	1.30	− 4	− 42
	25	1.94	1.80	− 7	− 35
	30	3.17	3.14	− 1	−100
30.0	10	0.71	0.71	0	− 13
	15	1.07	1.14	+ 7	− 16
	20	1.50	1.75	+17	− 36
	25	2.14	2.44	+14	− 28
	30	3.23	3.50	+ 8	− 15
50.0	10	0.87	0.74	−15	− 17
	15	1.31	1.26	− 4	− 24
	20	1.96	1.77	−10	− 40
	25	2.85	2.64	− 7	− 52
	30	4.44	3.81	−14	+ 13
75.0	10	0.88	0.85	− 3	− 17
	15	1.79	1.71	− 4	+ 13
	20	2.87	2.89	+ 1	+ 15
	25	3.91	4.22	+ 8	− 8
	30	5.08	4.92	− 3	+ 36

Each section of the figure comprises five separate pairs of graphs, a pair for each of the five maintained temperatures tested. All are plotted on the same scales. Abscissas represent oxygen pressure in

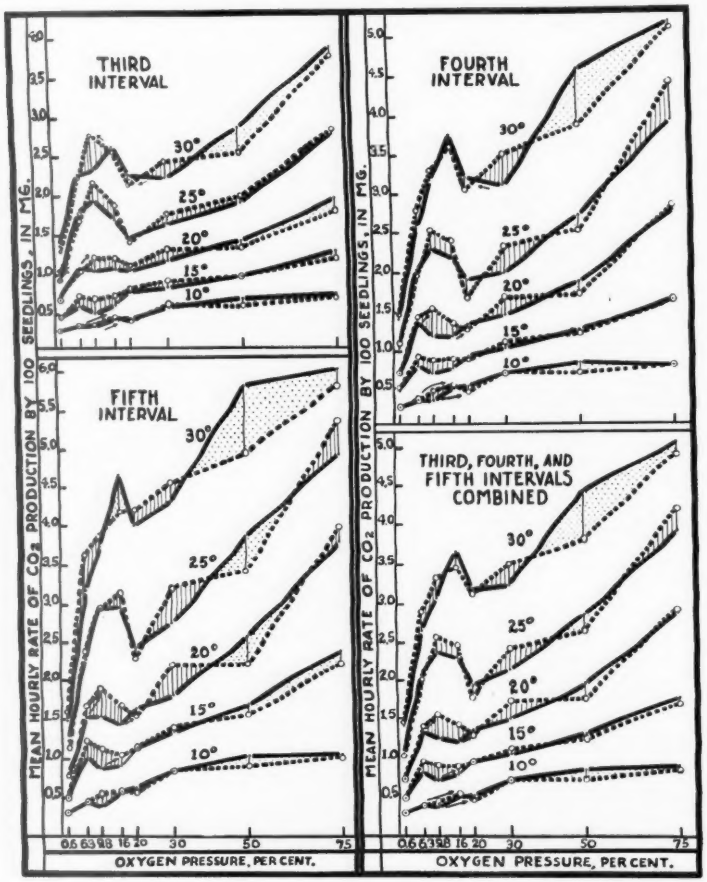

FIG. 1.—Oxygen-pressure graphs of CO_2 production in 3rd, 4th, and 5th intervals separately and combined, at five maintained temperatures, without ethylene (continuous lines) and with ethylene (broken lines). Arrows mark abscissal differences of critical points between control graphs and graphs for series with ethylene; hachured areas indicate ethylene acceleration of CO_2 production and stippled areas indicate ethylene retardation. Data are from tables III, IV, V, and VII.

the gas stream, from 0.6 to 75.0%, and ordinates represent the average hourly rates of CO_2 production. In each pair of graphs, the continuous line represents tests without ethylene (controls) and the broken line represents tests with ethylene. Positive ethylene influence (acceleration) is of course indicated where the broken line is above the corresponding continuous line (hachured areas); negative influence (retardation) is indicated where the broken line is below (stippled areas); and absence of any ethylene effect is indicated where the two graphs of a pair intersect. No smoothing has been applied to these graphs, to introduce presumable curvatures in place of straight-line segments and angles; only data that are actually available are represented. The new contribution on CO_2 production is shown by the broken-line graphs and their relations to the corresponding continuous lines.

The relations brought out by the several sets of graphs for the separate observation intervals are so nearly alike, and such differences as there are among the sets are generally so inconsistent, that it is concluded that time (or developmental phase) was apparently not an effective variable in this study. The discussion will therefore refer mainly to the data for the last three intervals combined.

MACK'S DOUBLE OPTIMUM OF OXYGEN PRESSURE FOR CO_2 PRODUCTION.—MACK (**32**) has already pointed out that this series of tests without ethylene showed not only the main oxygen-pressure optimum for CO_2 production (about 90–95%) but also a secondary optimal pressure with a very much lower value (between about 6.3 and about 16%, according to temperature). He has noted also that the latter value tended to be greater with higher temperature. MACK'S secondary optimal pressure is of course the abscissa of the first graph maximum on any of the continuous-line graphs of figure 1. A typical illustration is the continuous-line graph for 25° and for the last three intervals combined. That graph starts, at the left, with a very low ordinate (first graph minimum) for oxygen pressure of 0.6%, ascends to the first maximal point (with abscissa of 9.8%, the secondary optimum just mentioned), reverses and descends to a second minimal point with abscissa of 20%, and then reverses again and ascends toward the primary or main graph maximum, shown by MACK'S data as having an abscissa of 90–95%. The presence of the

second minimum naturally involves two critical abscissas, the ordinate for the first (first graph maximum) being greater than that for the second (second graph minimum).

The control graphs (continuous lines) of figure 1 generally agree in showing these two critical points, and their configuration for any temperature appears to be the same for all three intervals here considered, as well as for the last three intervals combined. That is true for the ethylene-treatment graph also (broken lines of figure 1), excepting those for 10° and 30° in the 5th interval. MACK'S evidence for the presence of the double optimum was based on just the data from the experiment series without ethylene, and the fact that these data from the series with ethylene so consistently support his evidence in this connection renders that already convincing evidence much more convincing; for the experiment series with ethylene was carried out independently and after the other series had been completed, as has been said. Whatever the effect of ethylene may have been, its presence in the gas stream surely did not generally prevent the appearance of MACK'S double optimum of oxygen pressure for CO_2 production.

The secondary optimum is of course least clearly evident on the graphs for the lowest temperature, for which all ordinates are low, and the difference in ordinate magnitude between the first graph maximum and the second graph minimum is greatest for temperatures of 25° and 30°. This difference is generally at least as great for the broken-line as for the continuous-line of the same graph pair. For both the control series and the series with ethylene, the abscissa of the first graph maximum (which is MACK'S lower or secondary optimum of oxygen pressure for CO_2 production) varied between 6.3 and 16%, and there appears to be a tendency for this critical pressure to be somewhat greater with higher temperature. For 10° and 20° it is greater with ethylene than without ethylene, but for the other tested temperatures it is alike for both series. In general, the second graph minimum has an abscissa extending 4–10 percentage units beyond the corresponding abscissa of the first graph maximum. Shiftings of the abscissal positions of these two critical points, as apparent ethylene effects, are indicated in figure 1 by small arrows pointing from a maximum or minimum on a continuous-line graph toward

the corresponding critical point on the adjacent broken-line graph. With further study these shiftings may prove to be of physiological significance, but the limitations of our data make it unnecessary to attempt here any reasoned explanations of them. For the present we may be content with the general statement that MACK's secondary oxygen-pressure optimum for all tested temperatures is shown as between 6.3 and 16% for both the control tests and those with ethylene, while the second critical point (second graph minimum) is shown for all tested temperatures as having an abscissa between 9.8 and 20% for both series. The abscissal difference between these two points of inflection may be as small as 4 percentage units or as large as 10.

For the series without ethylene, as MACK has shown, the main oxygen pressure optimum is shown as 90 or 95%; this statement covers all tested temperatures. Pressure of 98.3% was clearly superoptimal for CO_2 production when ethylene was not present.

MINIMAL AND MAXIMAL OXYGEN PRESSURES AND TEMPERATURES.—Our series of oxygen pressures and of temperatures were neither of them sufficiently extended to show any temperature-oxygen combinations preventing the elimination of CO_2. From the nature of the respiratory process, it seems safe to suppose that no minimal oxygen pressure for CO_2 production (at least with our background conditions) might be found for any temperature in either series. The initial slopes of the oxygen-pressure graphs of figure 1 all suggest pressure minima below zero, which of course refers to truly anaerobic production of CO_2. In a similar manner, it is safe to suppose that any maximal oxygen pressures that might perhaps be possible would surely be greater than 100% without ethylene, and probably also with ethylene. It will be remembered that the series without ethylene extended to a pressure of 98.4%, which is clearly superoptimal but still gave very rapid rates of CO_2 production (**32**). It must always remain logically impossible to test any oxygen pressure considerably above 100% without at the same time introducing a corresponding alteration in the background complex of our study, which involves a total gas pressure of about one atmosphere; the parameter limits of the investigation definitely preclude the employment of oxygen pressures higher than about one atmosphere. It is of course

logically possible, so far as our experiments go, that a maximal oxygen pressure for CO_2 production with ethylene might be found between 75 and 100%, for no oxygen pressures in that range have been tested with ethylene; but many considerations make such a supposition highly improbable.

With reference to temperature limits of CO_2 production, it is clear that the minimal temperature for appreciable maintenance of this process, either without ethylene or with it, must lie considerably below our lowest temperature (10°) for every tested oxygen pressure, probably very far below that temperature and perhaps well into the negative region of the thermometer scale. The maximal temperature for CO_2 production with any oxygen pressure, in either series of experiments, is obviously shown as above our highest tested temperature (30°), probably far above. But in this we are led, again by general considerations, to the thought (already familiar in the literature) that temperature maxima for this process are practically inconceivable; for CO_2 production is well known to continue indefinitely with higher temperature after a lethal temperature has been surpassed.

HIGHEST RATES OF CO_2 PRODUCTION.—The temperature-oxygen combinations that gave the highest rates of CO_2 production in both series of tests were those of high temperature and great oxygen pressure. Without ethylene the optimal combination is shown (**32**) as 30° and 95%, with a mean hourly rate (for 100 seedlings in the last three observation intervals) of 5.86 mg. The two corresponding rates nearest to this one were given by 30° combined with 90% (5.75 mg.) and with 98.3% (5.31 mg.). The combinations of 25° with 95, with 90, and with 98.3% gave the three next lower rates (4.87, 4.51, and 4.52 mg., respectively). For combinations including only oxygen pressures of 75% and below (for which range we have data from both the control series and the series with ethylene), the four highest rates of CO_2 production (for the last three intervals) and the combinations that gave them are: 5.08 mg. (30° and 75% without ethylene); 4.92 mg. (30° and 75% with ethylene); 4.44 mg. (25° and 50% without ethylene); 4.22 mg. (30° and 75% with ethylene).

Derived graphs of ethylene effect on CO_2 production

The graphs of figure 1 show that the presence of ethylene in the gas stream accelerated CO_2 production in some tests, retarded it in others, and exerted no considerable influence on this process in still other tests. It is also obvious that the nature of the ethylene effect was related to oxygen percentage in a definite way, while its absolute amount was related to oxygen percentage, temperature, and time. (The main oxygen-pressure relations of this ethylene effect were briefly presented by Livingston and Mack before the American Society of Plant Physiologists at New Orleans, December, 1931). A convenient way to bring out the relations of apparent ethylene effect to time, temperature, and oxygen pressure is to employ the plus and minus percentages shown in the last columns of tables II–V. These are our indices of ethylene effect, which will next be considered with respect to both sign and magnitude. Sufficient reduction of a positive value of course leads to the zero value, and still further reduction gives progressively *increasing* negative values; considered in this way, acceleration and retardation are brought into a single continuous series of magnitudes.

Comparison of corresponding values of the index of ethylene effect upon CO_2 production in successive observation intervals shows no consistent variation with respect to time (that is, to the advancing development of the seedlings). We shall consequently refer here, as before, only to the data for the last three intervals taken together. These are shown in table VII and by the continuous-line graphs of figure 2. The plus and minus percentages are ordinates in both parts of that figure. The left-hand part consists of five graphs, one for each tested temperature, and abscissas are oxygen-pressure values. The right-hand part consists of eight graphs, one for each tested oxygen percentage, and abscissas are temperature values. Each graph is accompanied by a horizontal line to represent the zero value of this index, positive values (acceleration of CO_2 production) being of course plotted above the line while negative values (retardation) are plotted below it. Only the scale of abscissas is shown, at the bottom of each part of the figure, but the ordinate value for each

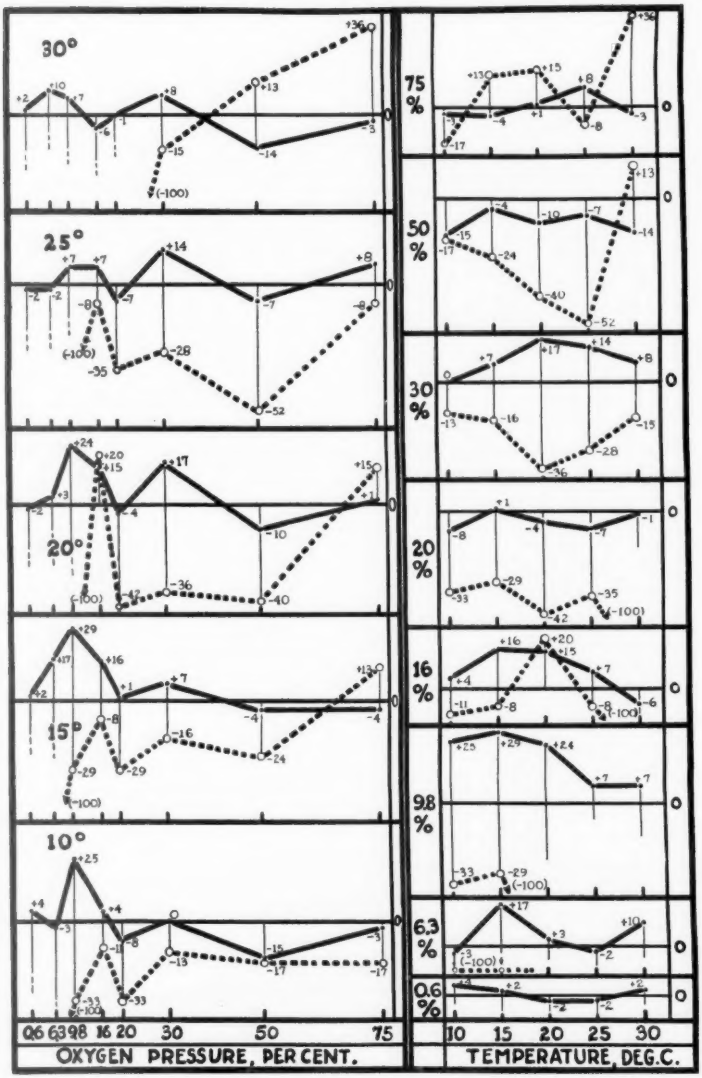

FIG. 2.—Plus and minus percentages of ethylene effect on CO_2 production (continuous lines) and shoot elongation (broken lines). Ordinate values are inscribed on the graphs. At left, five pairs of oxygen-pressure graphs; at right, seven pairs of temperature graphs. A common zero-line is shown for each pair. Data are from table VII.

point is inscribed near the point. Like ordinate scales are used for all graphs. (The broken lines of figure 2 represent percentage effects of ethylene on shoot elongation, to which reference will be made farther on.)

On the oxygen-pressure graph for 20° (left-hand part of figure 2), which may be taken as a typical example, ethylene effect is shown as negligible (−2%) for the lowest oxygen pressure (0.6%). The graph ascends and reaches a first maximum (+24%) for oxygen pressure of 9.8%. It then descends to a second minimum, showing a slight retardation (−4%) for oxygen pressure of 20%. It then ascends to a second maximum, with acceleration of +17%, for oxygen pressure of 30%. It descends again to a third minimum, for oxygen pressure of 50%, with retardation of −10%; and it ends with the point for oxygen pressure of 75%, for which the ethylene effect is shown as negligible (+1%).

The corresponding oxygen-pressure graphs for the remaining four temperatures are shaped essentially like the one for 20°, just described, but some quantitative differences that may be definitely related to temperature may be mentioned. The first maximal point has the abscissa 9.8 for 10°, 15°, and 20° but its abscissa may be somewhat larger for 25° and it is shown as 6.3 for 30°. The second minimal point has the abscissa 20 for 10°, 15°, 20°, and 25° but its abscissa is shown as 16 for 30°. The second maximal point is shown with the abscissa 30 for all five temperatures. The abscissa of the third minimum is shown as 50 for all temperatures excepting 15°, for which evidence is inconclusive. Whether the final point, with abscissa 75, is to be regarded as a third maximum is of course not clear, since available data do not extend beyond the oxygen pressure of 75%, but that point is considerably higher on the scale of ordinates than is the third minimum, in all instances excepting the graph for 15°, on which these two ordinates are alike (−4%).

Considering that the numerical results are surely not free from deviations, and considering the remarkable similarity among all the oxygen-pressure graphs of ethylene effect on CO_2 production (of which the continuous lines at the left of figure 2 are a summary), there seems to be no doubt that this remarkably consistent and regular relation between ethylene effect and oxygen pressure must be

regarded as a very real thing, at least within the parameter limits of this study. The effect produced by the introduction of ethylene into the environment of our cultures was clearly dependent on the current pressure of oxygen in the gas stream. For all temperatures tested ethylene treatment was generally *without considerable effect* with oxygen pressure about 0.6%; it generally gave pronounced *acceleration* with oxygen pressure about 9.8%; it generally produced some *retardation* (or no considerable acceleration) with oxygen pressure of about 20%; it generally gave considerable *acceleration* with pressure of about 30% and a similar *retardation* with pressure of about 50%; with oxygen pressure of about 75% the effect was generally negligible.

So far as we are aware, no such relation as this has hitherto been described in published accounts, although this relation is obviously in part somewhat nearly parallel with MACK'S (**32**) double optimum relation between oxygen pressure and CO_2 production by these seedlings in the absence of ethylene, which relation is now broadened to apply also to the tests with ethylene. The first maximum and the second minimum on a primary oxygen-pressure graph (fig. 1) have about the same abscissas, respectively, as are shown for the first maximum and the second minimum on the corresponding secondary oxygen-pressure graph of ethylene effect on CO_2 production (continuous lines, figure 2, left-hand part). This correspondence is not precise, but we may say, in general, that oxygen pressure of about 9.8%, which was specially suited to CO_2 production in both series, was also specially suited to the development of ethylene acceleration of that process; while pressure of about 20%, which generally allowed only low rates of CO_2 production in both series, also allowed only negligible ethylene effect.

No generalization that would apply to all oxygen pressures seems to be possible with regard to the manner in which ethylene effect on CO_2 production varied with temperature. Reference to the continuous-line temperature graphs of figure 2 (right-hand part) indicates that: (a) Temperature appears to have had little or no influence on ethylene effect for the lowest and highest oxygen pressures (0.6 and 75%), also for the intermediate pressure of ordinary air (20%). (b) With pressure of 50% all tested temperatures show negative effects (ethylene retardation of CO_2 production), but these are large

only for 10°, 20°, and 30°. (c) With pressure of 9.8% the effect is shown as positive (ethylene acceleration of CO_2 production) for all tested temperatures, but it is very great for 10°, 15°, and 20° and small for 25° and 30°. (d) Pressures giving different kinds of ethylene effect according to temperature are: 6.3% (negligible for 10°, 20°, and 25°, positive and great for 15°, positive and considerable for 30°); 16% (about negligible for 10° and 30°, positive and great for 15° and 20°, positive and small for 25°); and 30% (as for 16% but with greatest effect shown for 20° and 25° instead of 15° and 20°).

Considering degrees of ethylene acceleration of CO_2 production, it appears that the temperature range giving the greatest acceleration for any oxygen pressure tended to shift upward on the thermometer scale as oxygen pressure was greater. The greatest acceleration percentage (24–29%) is shown for 10°, 15°, and 20° with oxygen pressure of 9.8%, but marked acceleration (14–17%) is shown for 15° with pressure of 6.3%, for 15° and 20° with pressure of 16%, and for 20° and 25° with pressure of 30%.

Considering degrees of ethylene retardation of CO_2 production, no very pronounced negative effects are shown excepting for oxygen pressure of 50%, with which the greatest retardations amounted to from 10 to 15%. But it seems to be very significant that the oxygen pressure of ordinary air (20%) showed no considerable ethylene acceleration of this process at any temperature, and did show some small but probably considerable retardations at 10°, 20°, and 25°.

Many rather inadequately planned tests of ethylene effect on plant processes have been performed from time to time, by numerous experimenters, usually with ethylene mixed with ordinary air, and it is therefore interesting to note that our results show no very marked acceleration of CO_2 production with such a gas mixture. Had our tests, with 0.1% of ethylene in the gas stream, been confined to an oxygen pressure of 20% and to the ordinary temperatures of 20°–25°, our conclusion would probably have been that ethylene produced no acceleration of CO_2 production and seemed to retard it more or less, as much as 7% at 25°. Such a conclusion would of course be true for the special conditions referred to, but would be very far from representing the general relations of ethylene to CO_2 production in these seedlings.

Experiments performed with inclosed and stagnant bodies of gas whose constitution with regard to oxygen and ethylene is known only for the beginning of the exposure period may perhaps be of some value in the empirical development of ethylene treatments for horticultural or commercial purposes (that is, to hasten or modify the ripening, etc., of plant products or to promote the sale of ethylene), but such naïve and "practical" experiments can surely lead to nothing but confusion when scientific interpretation is attempted on the basis of rule-of-thumb procedures derived from such experimentation. Commercial and practical methods for the ethylene treatment of plant material certainly need to be developed and improved *ad hoc*, but the outcome of the limited study here reported indicates that we must await the development of fundamental science before useful rationalization concerning the complex causal influences involved here may be applicable at all. The present report is of course no more than a very simple and small beginning; further studies, with suitable plant material, adequate controls, and sufficiently precise experimental and logical technique will doubtless broaden our field of vision in this direction and may be expected to reveal some of the shortcomings of the present study.

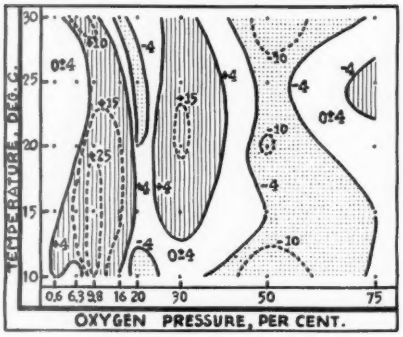

FIG. 3.—Three-dimensional diagram showing plus and minus percentages of ethylene effect on CO_2 production. Unshaded areas represent negligible effect (percentages between -4 and $+4$); stippled areas represent retardation (percentages below -4); hachured areas represent acceleration (percentages above $+4$). Data are from table VII.

The plus and minus percentages of ethylene effect on CO_2 production in the last three intervals are set forth in another way by the three-dimensional diagram of figure 3, which shows the relations of ethylene effect to both oxygen pressure and temperature. In the plane of the paper, vertical coordinates are temperatures and hori-

zontal coordinates are oxygen pressures, while the percentage indices of ethylene effect are represented by coordinates supposed to rise perpendicularly from the plane of the paper. The locations of the points of observation are shown by dots and the included plane surface is subdivided by contours (after the fashion of a topographic chart) into three kinds of areas. The regions without shading represent approximately negligible values of ethylene effect (from -4 to $+4$), the regions marked by stippling represent retardation (index values below -4), and those marked by hachures represent acceleration (index values above $+4$). Comparison of this diagram with the two-dimensional graphs of figure 2 and with the statements derived from them will help to show the complex relations that are indicated; of course these two figures show the same relations, for they are merely different plottings of the same set of numerical data.

Primary graphs of shoot elongation

The average values for shoot elongation, that is, the mean length of the longest ten shoots at the end of each test, are shown graphically in figure 4, the numerical data being taken from table VI. Continuous lines represent the tests without ethylene and broken lines represent those with ethylene. Ordinates are the growth averages in question and their numerical values are inscribed near the points of observation as plotted. The left-hand part of the figure consists of five pairs of graphs, a pair for each tested temperature, and abscissas are oxygen pressures. At the right of each control graph a short segment is added to show the approximate slope beyond the point for oxygen pressure of 75%, these indications being derived from MACK's earlier paper. The right-hand part of figure 4 consists of seven pairs of graphs, a pair for each tested oxygen pressure, and abscissas are temperatures. Although the growth data are derived from measurements much less precise than were the measurements of CO_2 production, yet the consistency of the several graphs of this figure indicates a high degree of reliability.

The continuous-line graphs, for the control series, without ethylene, show definite relations between shoot elongation, oxygen pressure, and temperature, as MACK has already pointed out. With the exception of the one for 30°, each of the oxygen-pressure graphs for

the control series (continuous lines, left-hand part of figure 4) shows a maximal point or region with abscissa about 50%. This is MACK's lower or secondary pressure optimum for growth. A first minimal

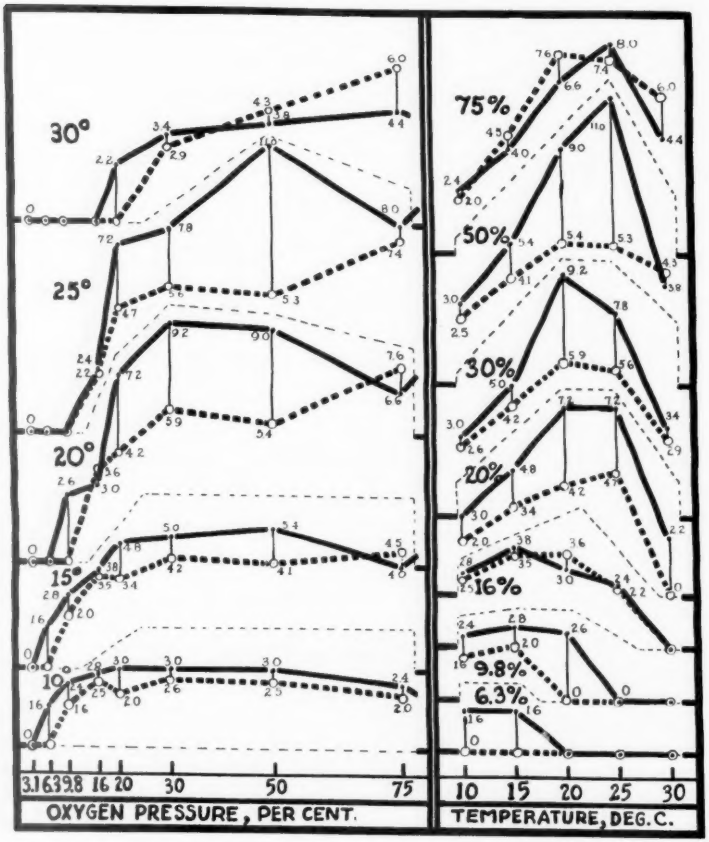

FIG. 4.—Graphs of shoot elongation without ethylene (continuous lines) and with ethylene (broken lines). At left, five pairs of oxygen-pressure graphs; at right, seven pairs of temperature graphs. A common zero-line is shown for each pair and the index values are inscribed on the graphs. Data are from table VII.

point (with index value of zero) appears at the left end in every case and this point shifts to the right with higher temperature.

Excepting on the graph for 30°, the ordinate at the extreme right (for pressure of 75%) is smaller than that for pressure of 50%, and MACK's data show that this point for pressure of 75% is a second graph minimum on the graphs for 15°, 20°, and 25°. The upper or main oxygen-pressure optimum for shoot elongation is 95%.

Each of the broken-line graphs shown in the left-hand part of figure 4, for the series with ethylene, lies generally below the continuous line of the same pair, which shows that the presence of ethylene in the gas stream, at a maintained pressure of 0.1%, retarded shoot elongation in nearly every instance. These differ from the corresponding continuous lines in that they fail to indicate either the first graph maximum or the second graph minimum. The latter is perhaps suggested by the graph for 10°, but the slope is generally upward throughout. The ethylene-treatment graphs for 10° and 15° each show what may perhaps be a significant sag in the region of pressure about 20%, a sag that is not shown by the corresponding control graphs nor by the other oxygen-pressure graphs for ethylene treatment. The graph pairs for 20° and 25° show exceptionally great ethylene retardation for oxygen pressures of 20, 30, and 50%, and the two graphs of each of these pairs consequently diverge widely in the region of these three pressures.

Without ethylene, the oxygen-pressure range that gave greatest growth at any tested temperature (fig. 4, left-hand part) tended to have higher pressure limits as the temperature was higher, but this cannot be said of the series with ethylene. As has been noted, MACK's secondary pressure optimum for growth failed to appear when ethylene was used, and the greatest pressure tested with ethylene (75%) gave, at each tested temperature excepting 10°, the greatest growth index for the given temperature. At 10° the optimal pressure range is shown as 30–50% without ethylene, but both graphs for this temperature may perhaps be regarded as nearly horizontal throughout the whole range from 16 to 75%. What might have been shown by ethylene treatment with oxygen pressures above 75% is of course not brought out.

The lowest tested oxygen pressure that gave measurable growth with ethylene at any tested temperature also tended to be greater as the temperature was higher, and this critical pressure with ethyl-

ene was generally one interval higher on our pressure scale than was the corresponding lowest pressure showing measurable growth without ethylene. The graphs for 25° are an exception to this statement, however, for 16% was the lowest tested pressure permitting measurable growth at that temperature, with ethylene as well as without ethylene. With that exception, the minimal oxygen requirement for shoot elongation was evidently greater with ethylene than without it and greater at higher than at lower temperatures.

No maximal oxygen pressure for shoot elongation at any tested temperature is shown for either the control series or the one with ethylene. If such a maximal pressure might be found it would probably be above the pressure of 100%, for both series and for all five temperatures,—surely above 75% for the series with ethylene and above 98.3% for the series without ethylene. If we suppose a maximal pressure for growth as above 100% (pure oxygen), the same logical difficulties are introduced as arose from the query about maximal oxygen pressures for CO_2 production.

Turning to the temperature graphs (right-hand part of figure 4), the temperature optimum for shoot elongation tended, in both series, to be higher as the oxygen pressure was greater. For pressure of 9.8% it is shown as 15° for both series. For pressure of 16% it is shown as 15° for the control series and as 15°–20° for the series with ethylene. For greater pressures it is shown for both series as 20° or 25°, or between these temperatures.

The lowest temperature to give measurable growth with ethylene was evidently below 10° for all tested oxygen pressures from 9.8 to 75% inclusive. Pressures of 0.6 and 6.3% failed to permit measurable growth with ethylene at any tested temperature, and it will be recalled from MACK's report that oxygen pressures of 0.6 and 3.1% without ethylene failed in the same way.

The highest tested temperature permitting measurable growth was evidently above 30° for all tested oxygen pressures greater than 16% without ethylene, and for all tested pressures greater than 20% with ethylene. For smaller pressures this highest temperature was lower as the pressure was smaller. It was also generally lower with ethylene than without it. For 20% it was 25° with ethylene but above 30° without ethylene; for 16% it was 25° for both treatments;

for 9.8% it was 15° with ethylene and 20° without ethylene; with 6.3%, 10° and 15° gave some growth without ethylene but neither of these temperatures nor any other tested temperature gave any measurable growth with ethylene; for 0.6% no tested temperature permitted measurable shoot elongation either with ethylene or without it.

With regard to the absolute values of the indices of shoot elongation, the highest index here considered is 11.0 (for 25° and 50%) in the control series, but the highest value in the series with ethylene treatment is only 7.6 (for 20° and 75%). Of course the lowest index is zero in both series, and many combinations of temperature and

Without ethylene			With ethylene		
Serial no.	Growth index	Combination	Serial no.	Growth index	Combination
1	11.0	25°, 50%	1	7.6	20°, 75%
2	9.2	20°, 30%	2	7.4	25°, 75%
3	9.0	20°, 50%	3	6.0	30°, 75%
4	8.0	25°, 75%	4	5.9	20°, 30%
			5	5.6	25°, 30%
5	7.2	20°, 20% 25°, 20%	6	5.4	20°, 50%
			7	5.3	25°, 50%
			8	4.7	25°, 20%
			9	4.5	15°, 75%
			10	4.3	30°, 50%
			11	4.2	20°, 20% 15°, 30%

oxygen pressure that gave no measurable shoot elongation with ethylene gave considerable elongation in the absence of that compound. If for each series we consider as "good" for shoot elongation the combinations whose indices fall in the upper third of the total index range for the series, we find that there are four such good combinations for the control series while there are seven for the series with ethylene treatment. The two "good" index ranges do not overlap, however; 8.0 is the smallest growth index in the "good" group without ethylene and 7.6 is the greatest index in the other "good" group. These "good" combinations are shown above, arranged in the descending order of their indices (whose values are also

shown), and each series is continued to include the combinations 20° with 20% and 25° with 20%, which are specially interesting because they may be most frequently encountered in nature at times when plants are active. These are shown in bold-face type. Indices below the horizontal line fall in the "medium" class of their own series; that is, in the middle third of the index range. The four "good" combinations without ethylene occur also among the "good" ones with ethylene, but with marked shifting of their order, and the three additional "good" combinations in the series with ethylene all fall in the "medium" class in the control series. The highest index for oxygen pressure of 20% (that of ordinary air) without ethylene is 7.2 (20° and 25°), and the corresponding highest indices for the same pressure with ethylene are 4.7 (25°) and 4.2 (20°). These combinations fall in the "medium" class in both series.

It is surely significant that, with respect to shoot elongation, higher oxygen pressures so greatly surpassed the pressure of ordinary air, both with and without ethylene. With respect to the rapidity of shoot elongation in the first few days after the beginning of germination, our lot of wheat seed was apparently well suited to temperatures frequently occurring naturally but it was apparently not well suited to any oxygen pressure ever encountered in nature; natural atmospheric oxygen pressure might be considered as at least 60% deficient for these seedlings, so far as shoot elongation is concerned. Of course such a statement is based on and limited by the background conditions of these experiments.

Although the available data on shoot elongation with oxygen pressures above 75% (90, 95, and 98.3%) cannot be considered in our discussions of ethylene effect, it may be added here (**32,** table V) that the series without ethylene furnished just one index value higher than 11.0 (namely, 12.2 for 25° and 95%), and also five additional values below 11.0 but still in the "good" class.

Derived graphs of ethylene effect on shoot elongation and their comparison with corresponding graphs of CO_2 production

The plus and minus percentages that represent effects of ethylene on shoot elongation (tables VI and VII) are shown by the broken-line graphs of figure 2, which are so arranged that each one may

readily be compared with the corresponding continuous-line graph of ethylene effect on CO_2 production. The same zero line is used for both graphs of each pair. Ordinates are the percentages, whose numerical values are inscribed on the graphs, and abscissas are shown at the bottom.

As has been remarked, the ethylene treatment employed generally retarded shoot elongation, but acceleration of that process occurred in five instances; namely, for oxygen pressure of 16% combined with 20° (+20), for 50% combined with 30° (+13); and for 75% combined with 15° (+13), with 20° (+15), and with 30° (+36). All of the remaining tested combinations of temperature and pressure showed considerable retardation due to ethylene, the percentage index nearest to zero being −8, which appears for three different combinations. Aside from the ultimate value of −100, the numerically largest negative index is −52. The total range of these percentages is from −100 to +36.

The oxygen-pressure graphs (broken lines, left-hand part of figure 2) of ethylene effect on growth at 10°, 15°, 20°, and 25° are remarkably similar in form and many of their features are in close agreement with the corresponding graphs of ethylene effect on CO_2 production. To appreciate this it is desirable to neglect the zero line and consider a single scale of ordinates, as though −29 and +29, for example, were given as 0.71 and 1.29 respectively, acceleration being thus denoted by values greater than unity and retardation by values smaller than unity; the actual drawing would be the same as is shown in the figure, however. Each of these four graphs of ethylene effect on growth shows a first maximum for pressure of 16% and a second minimum for pressure of 20% (the *first* minimum being at the extreme left). A second maximum, for 30%, is at least generally indicated, as is also a third minimum, for 50%. A third maximum, for 75% or above, is clearly shown for all tested temperatures excepting 10°.

For the lower three temperatures the most striking difference between these graphs and the corresponding oxygen-pressure graphs for ethylene effect on CO_2 production (continuous lines, left-hand part of figure 2) is that the first graph maximum for effect on growth corresponds to a pressure of 16%, while the first graph maximum

for effect on CO_2 production corresponds to a pressure of 9.8 %. For the graph pair for 25° a similar difference is indicated.

Each of these pressure graphs of ethylene effect on shoot elongation shows a definite and pronounced minimum for pressure of 20%. There was apparently something about this oxygen pressure of ordinary air that generally made for characteristically low rates of CO_2 production (both with and without ethylene) and for characteristically low ethylene effect on both CO_2 production and shoot elongation. It is suggested, as has been noted, that this same pressure also made for a somewhat retarded rate of shoot elongation at 10° and 15° with ethylene, but this suggestion does not appear for shoot elongation without ethylene nor at the three higher temperatures with ethylene. When we say "low ethylene effect" here it is of course understood that any negative value of the index of this effect is considered as lower than any positive value, etc., and that a lowering of effect in this sense may *decrease acceleration*, may *change acceleration to retardation*, or may *increase retardation*.

Turning to the temperature relations of ethylene effect on shoot elongation, no clear generalizations are to be derived from a study of the temperature graphs (broken lines, right-hand part of figure 2); nor is there any apparent general correlation between these graphs and the corresponding ones of ethylene effect on CO_2 production. It should be noted, however, that ethylene retardation of growth was great at all temperatures with pressure of 20%, and that the two temperature graphs for that pressure (which is critical for several features of this study, as just mentioned) are both rather nearly horizontal and almost alike in their departures from the horizontal excepting at the extreme right (30°). Temperature apparently exerted no clearly consistent influence upon the effect produced by ethylene, either on CO_2 production or on shoot elongation. For the pressure 16%, 20° appears to have been optimal for ethylene effect on growth, and the same temperature lies in the optimal region for the corresponding ethylene effect on CO_2 production. For pressure of 30% the two temperature graphs are of similar form but reversed; the continuous-line graph for ethylene effect on CO_2 production ascends from 10° to 20° and then descends with higher temperatures (all ordinates being positive, excepting the one for 10°, which is

zero), but the broken-line graph descends to a minimum for 20° and then ascends (all ordinates being negative). For pressure of 50% ethylene retardation of growth increased with temperature up to 25°, but this pressure at 30° gave one of the few instances of growth acceleration and the continuous-line graph (for effect on CO_2 production) shows no consistent relation to the broken-line graph.

All of our data concerning ethylene effect on shoot elongation are plotted together on the three-dimensional diagram of figure 5, which may be compared with the corresponding diagram for ethylene effect

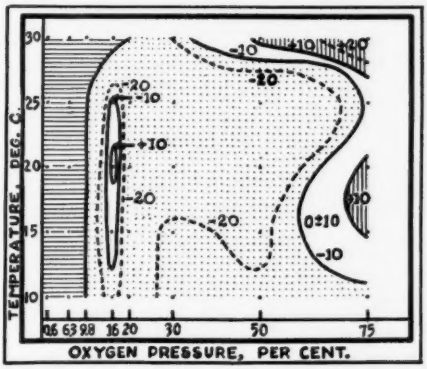

FIG. 5.—Three-dimensional diagram showing plus and minus percentages of ethylene effect on shoot elongation. Unshaded areas represent negligible effect (percentages between −10 and +10); stippled area represents retardation (percentages below −10); vertically hachured areas represent acceleration (percentages above +10). The horizontally hachured area represents combinations of temperature and oxygen pressure that permitted no growth either without ethylene or with it. Data are from table VI.

on CO_2 production (fig. 3) and with the two-dimensional graphs of figure 2. Unshaded areas represent percentage values between +10 and −10; that is, combinations of oxygen pressure and temperature that permitted little or no ethylene effect, either of acceleration or retardation. Vertically hachured areas represent ethylene acceleration of more than 10% and the stippled area represents ethylene retardation of more than 10%. The horizontally hachured area represents combinations that permitted no growth either without ethylene or with it.

It is of interest to note that the only tested combination of tem-

perature and oxygen pressure for which ethylene gave considerable acceleration of both CO_2 production and shoot elongation is the combination of 20° with oxygen pressure of 16%. With this combination the ethylene acceleration was 15% for CO_2 production and 20% for growth. But that combination gave only low indices of CO_2 production and shoot elongation, either without ethylene or with it.

It is apparent that current oxygen pressure was generally predominant in the control of ethylene effect upon both of the processes studied, as well as upon the rates of these processes themselves, and that maintained temperature was also influential, although not so regularly so. It is also apparent that the influence of ethylene on growth was intimately but not proportionately related to its influence on CO_2 production.

General conclusion

The experimental data presented in the preceding pages lead to the general conclusion that ethylene effects on our seedlings cannot be characterized in any simple manner; no definite statement about them can be made without quantitative reference to the concomitant non-ethylene conditions of oxygen pressure and temperature. It may easily be supposed that ethylene effects on living plants in general must be influenced not only by ethylene pressure, oxygen relations, and temperature relations, but also by water relations, nutrition relations, light relations, and the nature and physiological state of the tissues dealt with. Doubtless many chemical relations other than those mentioned may be influential on ethylene effects.

These remarks illustrate the more general and fundamental scientific principle of multiple causation, which is now belatedly becoming appreciated by many experimenters in physiology and other kinds of biological science. In experimental studies on the physiology of an organism it is obviously essential that real account be taken of all the influential conditions that are involved in the determination of process rates, through the definite establishment and specification of the experimental background complex as well as of the experimental variables that are considered.

Also, our results furnish an example of the manner in which what may be called second and third derivatives are now inevitably de-

manding attention in physiological studies of this sort. We shall need more and more to undertake studies on the manner in which effects, considered as due to a specified influence, may themselves be controlled through the action of still other concomitant influences. For instance, examples of possible accelerating or retarding influence of ethylene upon a plant process, such as CO_2 production or shoot elongation, are not very enlightening without reference to possible accelerating or retarding influence of current oxygen pressure upon the ethylene effects considered.

Summary

1. In this paper a review of literature on the influence of ethylene on plant processes is followed by an account of some preliminary experiments on ethylene effects. The rest of the paper reports a somewhat elaborate experimental study of the influence of temperature and oxygen pressure on the effects of a single maintained concentration of ethylene in controlling the rates of CO_2 production and shoot elongation in very young wheat seedlings. This study was carried out at the Laboratory of Plant Physiology of the Johns Hopkins University.

2. From the literature and from the results of the preliminary experiments, as well as from considerations of the nature and control of plant processes in general, it seemed that the many apparent discrepancies in observed ethylene effects on plants might be related to influences of non-ethylene conditions that must be effective along with ethylene influence. With this seemingly obvious, although generally unexpressed, thought in mind, an attempt was made to plan and carry out a relatively complete series of consistently comparable experimental tests. There were four experimental variables: maintained temperature, maintained oxygen pressure, time, and the presence or absence of ethylene at a single concentration or pressure. The background conditions of the environment, essentially alike for all tests, were established by using a standard 3-salt nutrient solution as culture medium (in which the seedlings were submerged), by the continuous bubbling of a synthetic gas mixture through the medium, and by the absence of light in the culture chambers. Nutrient solutions were not renewed. The gas stream maintained the

partial pressures of oxygen and nitrogen, or of these two gases and ethylene, and continuously removed CO_2 as well as any other volatile products of metabolism that may have been eliminated by the seedlings. There were 12 different gas mixtures without ethylene (containing 0.6–98.3% of oxygen by volume) and 8 different mixtures with ethylene (containing 0.6–75% of oxygen by volume). When ethylene was present its pressure was 0.1% by volume. Five different maintained temperatures were employed, 10°, 15°, 20°, 25°, and 30°. Five consecutive observation intervals in the experimental period gave five increments of CO_2 production in each experiment. Each experiment was repeated and some were several times repeated.

3. A relatively high degree of uniformity in the wheat seedlings used was secured by the employment of a standard germination procedure, with a 42-hour germination period, a maintained temperature of 19.5°, and a continuously flowing stream of air through water in which the seedlings were submerged; also by selecting the seedlings used out of a much larger number. One hundred seedlings were used in each test.

4. Carbon-dioxide increments were absorbed and measured by titration at the ends of the observation intervals and shoot elongation was measured as a single increment at the end of the entire experimental period. The experimental period was 48 hours, but the first 2 hours of it was allowed for seedling transfer and adjustment, and CO_2 measurements extended throughout the last 46 hours only.

5. The numerical results dealt with are: (a) average hourly rates of CO_2 production, for 300 different combinations of temperature, oxygen pressure, and time without ethylene, and 200 similar combinations with ethylene; (b) indices of shoot elongation for 60 different temperature-oxygen combinations without ethylene and 40 different combinations with ethylene, all these growth indices referring to the entire experimental period. These data are presented in tables and by means of graphs. Ethylene effects on CO_2 production and shoot elongation were computed as plus or minus percentages, on the basis of the corresponding controls without ethylene, and the resulting indices of ethylene effect are also presented in tables and graphs. Attention is given to the relations between ethylene effect on CO_2 production and ethylene effect on shoot elongation; as well as to the

influence of oxygen pressure, temperature, and time on ethylene effect.

6. The experiment series without ethylene was completed before the series with ethylene was begun and its main results have been previously reported by MACK (**32**). The new data presented in the present paper refer to the ethylene series, MACK'S data being employed as controls for the study of ethylene effects.

7. The more obvious relations apparently brought to light by this study may be summarized as follows:

Time not an effective variable with respect to CO_2 production within the limits of this study.—Relations between oxygen pressure and temperature on the one hand, and CO_2 production on the other, and the influence of ethylene on these relations, are most clearly shown by the data for the later observation intervals (when the seedlings were slightly more advanced in development), but there are no marked and consistent differences that can be related to time, aside from such differences as are connected with the larger CO_2 increments secured for the later intervals. Consequently the mean rates of CO_2 production for the last three observation intervals combined, which represent the last 36 hours of the experimental period, are mainly employed in our discussions.

Double optimum of oxygen pressure for CO_2 production.—MACK'S double optimum of oxygen pressure for CO_2 production is generally indicated for the experiment series with ethylene as well as for the series without it. This constitutes a pronounced confirmation of the reality of the double optimum, for the two series were independent but essentially alike with respect to oxygen pressures up to 75%, and they were planned to differ only with respect to ethylene.

Lower optimum of oxygen pressure for CO_2 production and the abscissal position of the corresponding secondary graph minimum.—A typical oxygen-pressure graph of CO_2 production ascends to a first maximal point (whose abscissa is MACK'S lower optimum of oxygen pressure), then descends to a second minimal point, and then ascends again toward the second maximal point (whose abscissa is the main pressure optimum). The abscissal positions of the first two of these critical points vary somewhat. It is suggested that these points tend to shift to the right (toward higher oxygen pressure) as the tem-

perature is higher and also as an apparent ethylene effect. The abscissa of the first graph maximum varies between 6.3 and 16% and the abscissa of the second graph minimum varies between 9.8 and 20%. The second minimum lies to the right of the first maximum by from 4 to 10 percentage units of oxygen pressure.

Main oxygen-pressure optimum for CO_2 production.—At all tested temperatures the main optimal oxygen pressure for CO_2 production was about 90–95% for the control series (as previously shown by MACK), and it is indicated as surely above 75% for the series with ethylene.

Minimal and maximal oxygen pressures and temperatures for CO_2 production.—No minimal or maximal oxygen pressure for CO_2 production appeared at any tested temperature, either without ethylene or with it; neither was any minimal or maximal temperature for CO_2 production shown for any tested oxygen pressure.

Optimal combinations of temperature and oxygen pressure for CO_2 production.—Without ethylene, the temperature-pressure combination that gave the highest rate of CO_2 production in the last three intervals together was 30° and 95%. That combination gave a mean hourly rate of 5.86 mg. for 100 seedlings. The only other combinations giving corresponding rates above 5.00 mg. were 30° combined with 90% (5.75 mg.) and 30° combined with 98.3% (5.31 mg.). For the series with ethylene, the highest mean hourly rate of CO_2 production in the last three intervals was given by the combination 30° and 75% (4.92 mg.). But the series with ethylene included no oxygen pressures above 75%, and if pressures higher than 75% are not considered in the control series, then the best combination for that series is also 30° and 75% (5.08 mg.).

Optimal oxygen pressures for shoot elongation.—For shoot elongation without ethylene there were generally two oxygen-pressure optima (as MACK has pointed out), a lower one about 50% and a higher one about 95%. Because the series with ethylene did not extend beyond the pressure of 75%, our data do not actually show the second of these pressure maxima for that series, although the probable occurrence of that maximum is indicated by the fact that pressure of 75% generally gave for any temperature a growth value higher than that given by any smaller pressure. The first or lower pressure op-

timum (about 50%) is not shown at any temperature for the series with ethylene.

Minimal oxygen pressures for shoot elongation.—The minimal oxygen pressure for shoot elongation at any temperature, which varied (for both series) from below 6.3% (10°, without ethylene) to about 20% (30°, with ethylene), was evidently greater at higher temperature than at lower, and also greater with ethylene than without it.

Maximal oxygen pressures for shoot elongation.—No maximal oxygen pressure for shoot elongation at any temperature is shown for either the control series (for which it was evidently above 98.3%) or for the series with ethylene (for which it was evidently at least above 75%).

Optimal temperatures for shoot elongation.—Both without ethylene and with it, the optimal temperature range for growth with any tested oxygen pressure was about 15°–20° for pressures of 9.8 and 16%, and about 20°–25° for pressures of 20% or above.

Minimal temperatures for shoot elongation.—The minimal temperature for shoot elongation was evidently below 10° for all tested oxygen pressures above 6.3%, both without and with ethylene, and also for 6.3% without ethylene. But no measurable growth occurred at any tested temperature for pressure of 0.6% in either series, nor for pressure of 3.1% without ethylene, nor for pressure of 6.3% with ethylene.

Maximal temperatures for shoot elongation.—The maximal temperature for shoot elongation was evidently above 30° for all oxygen pressures greater than 16% without ethylene and for all pressures greater than 20% with ethylene. For smaller pressures this critical temperature was lower as the pressure was smaller and generally lower with ethylene than without it.

Optimal combinations of temperature and oxygen pressure for shoot elongation.—For shoot elongation the best of the tested combinations of temperature and oxygen pressure without ethylene was 25° with 50%; this gave a growth index of 11.0. With ethylene the best combination was 20° with 75%, which gave an index of 7.6. The four combinations that gave largest growth indices (11.0 to 8.0) in the control series occur among the seven combinations that gave largest indices (7.6 to 5.3) in the series with ethylene. Each of the two index

ranges just mentioned embraces the upper third of the total range for its series. These seven "best" combinations are all for 20° or 25° combined with oxygen pressure of 30, 50, or 75%.

General effect of ethylene on CO_2 production.—The influence of ethylene on CO_2 production at any temperature varied in a remarkably complex but very consistent manner, according to the current oxygen pressure. At all tested temperatures it was *negligible* or nearly so for very small oxygen pressure (about 0.6%) and also for the greatest pressure tested with ethylene (75%). But the presence of ethylene generally *accelerated* CO_2 production for each of two intervening but widely remote ranges of oxygen pressure (about 10 and 30%), and it *retarded* this process, or else was without considerable effect, for the oxygen pressure of ordinary air (20%) and for oxygen pressure of 50%.

Optimal combinations of temperature and oxygen pressure for ethylene acceleration of CO_2 production and for ethylene retardation of the same process.—The most pronounced ethylene acceleration of CO_2 production (amounting to 29%) occurred with the combination of 15° with oxygen pressure of 9.8%, but the combinations of that same oxygen pressure with 10° and with 20° gave nearly as pronounced accelerations (amounting to 25 and 24% respectively). The most pronounced ethylene retardations of CO_2 production occurred with pressure of 50% combined with 10°, 20°, and 30° (amounting to 15, 10, and 14% respectively). With that same pressure of 50%, 15° and 25° showed smaller retardations (amounting to 4 and 7 % respectively). Retardations amounting to 7 and 8% were shown by oxygen pressure of 20% combined with 10° and with 25°.

Effect of ethylene on shoot elongation.—The influence of ethylene on shoot elongation was retardation for nearly all tested combinations of temperature and oxygen pressure, and the nature and degree of this influence was clearly related to the current oxygen pressure and to the concomitant influence of ethylene on CO_2 production. At 10°, 15°, 20°, and 25°, growth retardation was relatively small, or there was acceleration, for oxygen pressures of 16, 30, and 75%, and the growth index was generally relatively great for oxygen pressures of 20 and 50%. At 30° oxygen pressures of 20% and below failed to permit measurable growth with ethylene, but pressure of 30% gave retardation and pressures of 50 and 75% gave acceleration.

Oxygen pressure of ordinary air.—It is specially noteworthy that the oxygen pressure of ordinary air showed peculiar relations. It not only induced characteristically low rates of CO_2 production, both without ethylene and with it, but also gave most pronounced ethylene retardation of shoot elongation and little or no ethylene effect on CO_2 production.

Ethylene acceleration of growth.—Ethylene acceleration of shoot elongation occurred for 15° combined with oxygen pressure of 75%, for 20° combined with 16 and with 75%, and for 30° combined with 50 and 75%.

General temperature relations of ethylene effects on CO_2 production and on shoot elongation.—Although the temperature relations of ethylene effect on CO_2 production and on shoot elongation appear to be characteristically different for different tested oxygen pressures, yet no clear generalizations seem to be possible concerning these temperature relations; to appreciate this the graphs need to be consulted. Within the limits of our tests, oxygen pressure of 20% showed little or no influence of temperature upon ethylene effect, either upon CO_2 production or upon shoot elongation.

Ethylene acceleration of both CO_2 production and growth.—The only tested combination of temperature and oxygen pressure that gave considerable ethylene acceleration of both CO_2 production and shoot elongation is the combination of 20° with 16%. This acceleration amounted to 15% for CO_2 production and 20% for shoot elongation.

Generalization from main results.—For our seedlings and for the background conditions of our experiments, current oxygen pressure was generally predominant in the control of ethylene effect upon both CO_2 production and shoot elongation, as well as upon the rates of these processes themselves, either without ethylene or with it; current maintained temperature was also influential, although not so regularly so; and the influence of ethylene on shoot elongation was intimately but not proportionately related to its influence on CO_2 production.

General conclusion.—The results of this study furnish an illustration of the principle of multiple causation, according to which many influential conditions or effective factors may take part in the determination of physiological effects, which are often popularly attributed to some single influence. No scientifically useful conception

of ethylene influence on plants can be formed without the specification of the non-ethylene conditions that operate along with ethylene in each experimental test. In other words, these results illustrate how the influence of one condition may be modified through the concomitant action of other conditions.

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STRUCTURAL AND METABOLIC AFTER-EFFECTS OF SOAKING SEEDS OF PHASEOLUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 441

WILLIAM MARSHALL BAILEY

(WITH THREE FIGURES)

Introduction

The purpose of this investigation was to determine the influence which certain physiological conditions of the seed may exert upon subsequent development of the plant. The work was concerned mainly with the effects produced by soaking the seeds for a prolonged period in distilled water previous to planting them. KIDD and WEST first gave prominence to this problem by their experimental studies (**4**), and by an extensive review of the literature on related investigations (**5**–**8**). In this review were considered the effects of the conditions under which the parent plant had grown, the degree of ripeness, the conditions operating during germination and early seedling stages, and the effects of soaking the seeds in water and certain salt solutions in determining the subsequent course of development. The evidence in general seems to indicate that the factors which influence a plant during its earliest stages of development have a more or less pronounced effect upon its subsequent life history.

In their experiments, KIDD and WEST (**4**) soaked seeds under 4 cm. of distilled water at an average temperature of 17° C. for periods varying from 8 to 72 hours. Their report does not indicate that any provision was made for the elimination of bacteria or for aeration. The seeds used were *Phaseolus vulgaris* (dwarf bean), *Pisum sativum* (culinary pea), *Vicia faba* (broad bean), *Lupinus albus* (white lupine), *Helianthus annuus* (sunflower), *Triticum vulgare* (wheat), *Hordeum sativum* (barley), *Avena sativa* (oats), and *Brassica alba* (white mustard). The seeds were germinated on damp sand in porous flower pots covered with glass. The results showed that soaking the seeds more than 24 hours diminished the rate of germination in *Pha-*

seolus, Pisum, and *Helianthus,* and that there was a decrease in the rate of germination in *Hordeum* resulting from 72 hours of soaking.

They obtained data showing the effects of soaking seeds (dwarf bean, culinary pea, barley) on subsequent growth by growing them for 12 days on damp sand. They found that the soaking had a marked effect in retarding subsequent growth of the plants. They also carried on experimental work with seeds treated with distilled water and grown for longer periods in the greenhouse. Wheat, oats, white mustard, dwarf beans, broad beans, and white lupine were used. Samples of these seeds were soaked 6, 24, 48, and 72 hours respectively, and planted in potting soil in the greenhouse. In the case of dwarf bean harvested $4\frac{1}{2}$ weeks after sowing, a period of soaking of only 6 hours had a marked predetermining influence in retarding growth, which was greatly augmented by longer periods of treatment. Soaking for 3 days killed all of the seeds. Broad bean 3 weeks after planting showed diametrically opposite results, since there was a progressive increase in the height of the plants and in the rate of germination of the seeds resulting from the soaking, which was carried on up to 72 hours. Wheat showed a progressive increase in height and dry weight of tops in the plants from soaked seeds up to 48 hours of treatment. There was also a small increase in the rate of seed germination. However, plants from seeds soaked 72 hours were smaller and less vigorous. Oats showed results similar to those for wheat.

In addition, KIDD and WEST investigated the effects of temperature on the predetermining influence of soaking seeds of dwarf bean in distilled water (9). It was found that the temperature during the seed treatment had no marked effect upon the yield, but that the deleterious influence of soaking was somewhat less at temperatures near 20° C. than at temperatures higher or lower than this.

Three main conclusions were drawn from the results of this experimental work: first, that the soaking in distilled water previous to sowing may have a marked influence upon subsequent growth of the plants; second, that a germination test cannot be relied upon as a criterion of what this influence will be; third, that the nature of this influence is strongly specific. Thus very different results may be obtained from closely related plants. While soaking the seeds in water

greatly retarded the growth of the plants in the case of dwarf bean, this treatment increased both the rate of germination and the size of the plants produced in the case of broad bean.

TILFORD, ABLE, and HIBBARD (**19**), employing many kinds of seeds, found that deleterious effects are produced by prolonged water-soaking. Different results were secured from closely allied plants. A short period of soaking (8 hours) was found to interfere with germination of dwarf bean seeds and had a deleterious influence on subsequent development of the plants. The data submitted give results only on germination. In three tests sterilized seeds treated 72 hours in sterilized distilled water with aeration gave germination tests of 72, 100, and 88 per cent respectively. Non-sterilized seeds treated 72 hours in distilled water with aeration gave germination tests of 14, 40, and 27 per cent in three respective tests. Non-sterilized seeds soaked in distilled water 72 hours without aeration gave 20, 0, and 0 per cent of germination in three tests. It was concluded that the injurious influence on seed germination resulting from the soaking in distilled water is due to bacterial activity, and to a deficiency of oxygen and an excessive accumulation of carbon dioxide.

RHINE (**15**) found that soaking wheat for a period of 22 days in sterilized distilled water reduced the rate of germination to 2 per cent, while soaking for a period of 29 days resulted in a complete failure of germination.

BARTON (**1**), while experimenting on autolysis in seeds, investigated the influence of soaking for long periods of time on seed germination. Seeds of navy beans, peas, corn, and wheat were used. Four-ounce bottles, fitted with two-hole stoppers and right-angled glass tubes, were connected in series. The bottles and connections were sterilized. Each series of bottles was filled with boiling water. When at room temperature, the water was saturated with carbon dioxide. Five seeds, after being sterilized and washed with sterilized distilled water, were placed on glass wool in the first bottle of the series. After periods of soaking varying in length up to 3 months, the water was forced out with sterilized oxygen. In 3 or 4 days, 60 to 100 per cent of the seeds germinated. BARTON concludes: "(1) that the capacity for germination is not destroyed by long-continued soaking in sterile media; (2) that destructive changes are not brought

about by enzymes or ferments within the seeds; and (3) that any change that may take place within the seed does not in any way affect the viability and is not accompanied by any visible manifestation."

In the present investigation, the study of the predetermining influence of soaking seeds in distilled water has been carried further, in an effort to determine whether this influence is to be ascribed entirely to bacterial action and interference with respiration, or whether other causes are operating; and to discover some of the subsequent structural and metabolic effects of this seed treatment.

Experimentation

Early Valentine beans (*Phaseolus vulgaris*) were used in the investigation, seeds of relatively high germinating capacity being secured. The seeds were carefully sorted, only sound ones of approximately uniform size and regular shape being used.

The seeds were soaked in sterilized distilled water aerated by means of the apparatus shown in figure 1. In order to secure uniform movement of the air, it was drawn through the apparatus by means of an aspirator. The volume of air used was approximately 24 liters per day. It seems evident that lack of oxygen or the accumulation of carbon dioxide was not a limiting factor in this investigation, in view of the fact that TANG's (**18**) results indicated that 6 liters of air per day was sufficient to give maximum results in the germination of wheat. The distilled water in flask *A* was sterilized in the autoclave, the flask being plugged with cotton, and cooled to room temperature. The cotton filters F_1 and F_2, carefully wrapped in heavy paper, were sterilized by prolonged heating in the autoclave. The remainder of the apparatus was sterilized by heating for 2 hours in steam at the temperature of boiling water, it being impossible to use the autoclave because of injury to the rubber connections by the high temperature. After sterilization the apparatus was connected up with as much precaution as possible to avoid contamination with bacteria and fungus spores from the air.

The seeds were presoaked 10 minutes in distilled water (**2**), after which they were put in the seed chamber *S*, which was then filled with a 0.25 per cent solution of "uspulun," to sterilize the seeds.

It was found in preliminary tests that this treatment in no way impaired germination. After 15 minutes the uspulun solution was drawn over into flask *E*, and replaced with water from flask *A* by means of suction from the aspirator. Approximately 1.5 liters of water was drawn through the seed chamber from flask *A* for the purpose of washing the seeds. Air was then drawn steadily through the seed chamber, after being sterilized by passing through the cotton

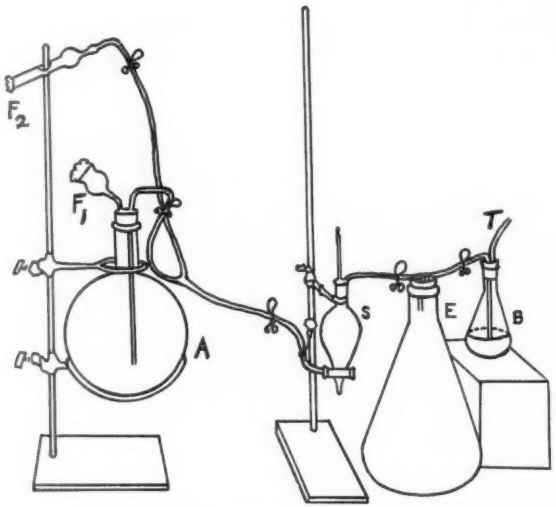

FIG. 1.—Apparatus for soaking seed with aeration: *S*, seed chamber; *A*, flask containing sterilized distilled water; *E*, large flask into which water was drawn from seed chamber; F_1, F_2, cotton filters; *B*, safety flask; *T*, tube connected to aspirator.

filter F_2. The water was changed four times per day by being drawn from flask *A* into the seed chamber *S* and into flask *E* by suction from the aspirator. When the water was all drawn from flask *A* it was replaced from time to time with flasks similarly filled with distilled water, sterilized and cooled, as much precaution as possible being taken to prevent contamination from the air in changing the flasks.

To test the success in the elimination of bacteria and fungi, a quantity of nutrient broth was put into a flask of the same size as

flask *E* and plugged with cotton. The flask with its contents was sterilized in the autoclave, cooled, and then connected with the apparatus in the place of flask *E*, as much precaution as possible being taken to avoid contamination from the air. A small quantity of the water in which the seeds had been soaking was drawn from the seed chamber into the broth. After incubation in a warm room for a period of 3 days, no indication of bacteria or fungi appeared in the broth. This test was made during the third day of the seed treatment.

In each experiment in which the seeds were planted, the seeds used for control were sterilized in the usual way, then washed as quickly as possible and planted at once. They are called "untreated seeds" in the report of the results.

The plants were grown in the greenhouse and were watered with the usual water supply available there. The temperature of the greenhouse usually varied between 60° and 80° F. The seeds were planted at a depth of approximately 1 inch. The plants were harvested when they had reached the blooming stage, when they were considered mature. The tops were harvested, the stems being cut at the surface of the soil.

Experiment I

The purpose of this experiment was to determine the effect of the seed treatment on germination, total number of mature plants produced, growth, average weight, time required to reach maturity, and influence of seed treatment on stem and leaf structure. The temperature at which the seeds were treated was 16°–19° C. The following seed treatments were used: no. 1, seeds untreated; no. 2, seeds treated 8 hours; no. 3, seeds treated 2 days; no. 4, seeds treated 5 days; no. 5, seeds treated 7 days; no. 6, seeds treated 9 days. This experiment was carried out between March 22 and May 9, 1930.

Stem and leaf structure.—Cross-sections of the stems in the internode just above the first pair of leaves were prepared from the mature plants. The average diameter of the stem and the average thickness of the xylem and phloem in this internode were determined in the plants from the untreated seeds and in those from seeds treated 5 days. Also the ratio of the average thickness of the phloem

to that of the xylem was determined. Cross-sections of leaves from the mature plants of these two sets were also prepared. For the sake of uniformity, the leaf sections were all taken from the terminal leaflet of the uppermost mature leaf. The leaf structures of the two sets of plants were compared.

EXPERIMENT II

This experiment attempted the determination of the influence of the seed treatment on the percentage of water and dry matter in the plants produced, the abundance of various carbohydrates and nitrogen compounds, and the influence on the growth of the plants of a calcium salt in very dilute solution in the water in which the seeds were soaked. The experiment ran from November 22, 1930 to January 20, 1931. The following were the respective seed treatments: no. 1, seeds untreated; no. 2, seeds treated in sterilized distilled water with aeration 5 days; no. 3, seeds treated in sterilized distilled water containing calcium nitrate (approximately 900×10^{-6} gm. normal per liter) with aeration 5 days. The temperature was 15°–16° C. The plants were grown in the greenhouse and harvested at maturity. The three sets of plants were harvested at the same hour of the day, 8 A.M.

Analyses for determining the percentage of dry matter, carbohydrates, and nitrogen compounds were made on samples of plants from no. 1 and no. 2. Determinations were made in duplicate. All analyses of the extractives were made within 3 weeks after harvesting. The Methods of Analysis of the A.O.A.C. (**13**) were generally followed in the chemical determinations.

EXTRACTION.—Not less than 20 plants (**14**) were ground together in a Russwin mill, and the ground material thoroughly mixed. Samples of this material were weighed off into sufficient 95 per cent alcohol, boiling hot, to make the alcohol content of the entire mass approximately 85 per cent. To this material was added 0.5 gm. of pure calcium carbonate to neutralize the acids present and thus prevent hydrolysis. The samples, in large pyrex beakers covered with watch glasses, were kept heated to the boiling temperature of the alcohol on the hot-water bath for 40 minutes. The samples were then extracted in Soxhlet extractors with 95 per cent alcohol, ether, and

water, each for 2–4 hours, then with 95 per cent alcohol over night. All alcohol used for preserving and extracting the samples was redistilled in glass, and only chemically pure ether was used. The alcohol, ether, and water used in the extraction were poured together into a large evaporating dish, and the alcohol and ether were replaced with water by evaporation. The extract was then transferred quantitatively to a volumetric flask and the volume adjusted by the addition of water. The lipoid materials, together with the chlorophyll, which were precipitated out by the replacement of the alcohol and ether with water, were removed in solution in 20 cc. of chloroform. The chloroform solution was then evaporated to dryness and the weight of the substances dissolved in the chloroform determined. Since this material consists mainly of chlorophyll and the carotinoid pigments, and very little difference was found between the percentages in the plants from treated seeds and those from untreated seeds, it was assumed that there was no important difference in the quantity of these pigments in the two sets of plants.

TOTAL WEIGHT OF SOLIDS.—The total dry weight of the solids was determined by taking the sum of the dry weight of the solid residue left after extraction, the solids of the extract computed from an aliquot evaporated to dryness and weighed, and the dry weight of the substances removed from the extract in solution in chloroform.

SUGARS.—In estimating reducing sugars, 50 cc. of the extract was measured off, cleared with basic lead acetate solution, and deleaded with potassium oxalate solution. The volume was then adjusted, and the reducing sugars of 50 cc. were computed as glucose by the Bertrand volumetric method, and calculated for the entire sample.

The non-reducing sugars were computed as sucrose. From the solution previously cleared and deleaded, 50 cc. was measured off and 5 cc. of HCl added. This mixture was heated for 1 hour on the hot-water bath and then set aside for 48 hours. It was then neutralized with sodium hydroxide and sodium carbonate, and the volume adjusted to 100 cc. The total reducing sugar was computed as invert sugar by the Bertrand volumetric method. The reducing-sugar content before hydrolysis was also computed as invert sugar. The difference between these multiplied by 0.95 gave the sucrose, which was computed for the entire sample.

POLYSACCHARIDES.—For the determination of the total polysaccharides, a small sample of the dry solid residue was weighed off into a Kjeldahl flask, and to this were added 200 cc. of water and 12.5 cc. of concentrated HCl. This mixture was boiled under a reflux condenser for 3 hours. The mixture was then cooled and neutralized with sodium hydroxide and sodium carbonate, cleared and deleaded, and its volume adjusted. The reducing sugar was estimated as glucose by the Bertrand method, and the weight of the polysaccharides was calculated by multiplying this result by 0.9. The weight of the polysaccharides was then calculated for the entire sample.

The starch was estimated from the results of saliva digestion, the method employed by CLEMENTS (3) in determining the starch content of leaves. To secure gelatinization of the starch, a small amount of the dry residue left after extraction was weighed off, put in a pyrex beaker, and moistened with water. After a few minutes 50 cc. of water was added. The mixture was then boiled for a moment over a gas burner, the inside of the beaker washed down, and the beaker, covered with a watch glass, transferred to the hot-water bath for 1 hour. The contents of the beaker were then cooled to approximately 37° C., and 10 cc. of filtered saliva was added. The mixture was incubated over night at 37° C. A check sample of the material was treated in the same way, and samples of this were tested with iodine and examined microscopically to make sure that the hydrolysis of the starch was complete.

After the starch digestion was complete, the mixture was cleared, deleaded, and its volume adjusted. From this, 100 cc. was taken and acidified to the extent of 2.5 per cent with sulphuric acid and heated on a boiling hot-water bath for 1.5 hours to hydrolyze to glucose the maltose resulting from the starch digestion. The solution was then neutralized with sodium hydroxide and sodium carbonate and its volume adjusted. The glucose was estimated by the Bertrand method and the starch calculated by multiplying this result by 0.9.

The hemicelluloses were determined by taking the difference between the total polysaccharides and the starch.

TOTAL CARBOHYDRATES.—The total carbohydrate content was determined by adding together the reducing sugars, sucrose, and total polysaccharides.

NITROGEN COMPOUNDS.—The total nitrogen of the extract was determined by the Gunning method modified to include the nitrogen of the nitrates. The organic nitrogen of the extract was determined by the Gunning method. For these determinations 50 cc. samples of the extract were dried in Kjeldahl flasks on the hot-water bath. The amino-acid nitrogen of the extract was determined by the Van Slyke method. The nitrogen of the solid residue was determined by the Gunning method. The weight of the protein was obtained by multiplying this result by 6.25. The total nitrogen of the sample was found by taking the sum of the total nitrogen of the extract and that of the solid residue.

EXPERIMENT III

In this experiment the respiratory carbon dioxide was determined by the method of double titration, and the rates of respiration in the treated and untreated seeds after sprouting were thus compared. The air was drawn into the respiratory chamber through a series of five wash bottles, the first four containing sodium hydroxide solution and the last barium hydroxide; and from the respiratory chamber through the gas absorption bottle (Milligan) containing 250 cc. of normal sodium hydroxide solution for the absorption of the respiratory carbon dioxide; then through a wash bottle containing barium hydroxide, and a safety flask connected with the aspirator by means of which the air was drawn through the apparatus at a uniform rate.

Two sets of seeds were used. In set no. 1 the seeds were untreated (presoaked, sterilized, and washed); in set no. 2 the seeds were presoaked, sterilized, washed, and soaked in sterilized distilled water with aeration in the usual way 3 days. The seeds of each set were sprouted in a germinator until the sprouts were approximately 2 cm. long before being put in the respiration chamber. Many of the seeds of set no. 2 did not sprout. Twenty-five sprouted seeds were used in each of the tests. These respiration tests were carried out at a temperature of approximately 25° C. They were made in duplicate, and were continued exactly 10 hours. At the end of this period the solution in the gas absorption bottle was titrated with normal and 0.1 normal sulphuric acid with phenolphthalein indicator, and then with 0.1 normal sulphuric acid with methyl orange indicator. After correction with blank tests on the normal sodium hydroxide, the second

titration gave the measurement of the respiratory carbon dioxide. As a result of the first titration, the carbon dioxide is held in the form of $NaHCO_3$. The carbon dioxide is set free in the second titration.

EXPERIMENT IV

In this experiment the effect on catalase activity of soaking seeds in distilled water both with and without aeration was investigated. For washing and soaking the seeds, distilled water sterilized in the autoclave and cooled in the ice-box was used. The seed treatment consisted of presoaking in distilled water 10 minutes, sterilizing with 0.25 per cent uspulun solution 15 minutes, washing with distilled water, and soaking in distilled water. After soaking the seeds were dried in the draft of an electric fan for 48 hours to the air-dry state (**12**). The untreated seeds were used in their original dry state, but were exposed to the draft of the electric fan with the soaked seeds in order to bring them as nearly as possible to the same degree of dryness.

The method employed by SHULL and DAVIS (**17**) was followed in the catalase tests, Oakland "dioxogen" being used. The tests were run in triplicate. Not less than 20 seeds were used in preparing the material for each test. The seed coats, hypocotyls, and plumules were removed. The cotyledons were finely ground in a glass mortar, and this material was sifted through a 100-mesh sieve. Of this material, 0.2 gm. was used in each test. In order to neutralize acids, 0.5 gm. of pure calcium carbonate was mixed with 10 cc. of the dioxogen in the funnel of the catalase apparatus. The percentage of dry matter in the pulverized material was determined, and the oxygen liberated by the catalase was calculated on the basis of the dry weight.

In experiment IVa, three sets of seeds were used. In set no. 1 the seeds were untreated; in sets 2 and 3 the soaking with aeration was continued 2 and 5 days respectively, the water being changed four times daily during the soaking. Each seed chamber was placed in a water bath and the temperature thus kept at 12°–14° C. The apparatus and method employed in soaking the seeds with aeration were those used in the preceding experiments (fig. 1). In experiment IVb also three sets of seeds were used. In set no. 1 the seeds were untreated; in sets 2 and 3 the soaking without aeration was con-

tinued 2 and 5 days respectively. The seeds were soaked in Erlenmeyer flasks of 500 cc. capacity, plugged with cotton, and previously sterilized in the autoclave. Precautions were taken to prevent contamination of the seeds with bacteria, and no indications of bacterial growth during soaking were seen. The water was not changed during the period of soaking. The seeds were kept in the ice-box at a temperature of 12°–14° C. during the soaking period.

Results

EXPERIMENT I.—The results of this experiment show a progressive decrease in the rate of germination resulting from the seed treatment. There is a still greater reduction in the number of mature plants produced, since a considerable number of them from treated seeds were unable to survive until they reached maturity. There is also a progressive decrease in the average weight of the tops, and an increase in the time required for the plants to reach maturity with the lengthening of the period of seed treatment. The results are shown in table I.

TABLE I

INFLUENCE OF SEED TREATMENT ON GERMINATION (APPEARANCE OF SEEDLINGS AT SURFACE OF SOIL), NUMBER OF MATURE PLANTS PRODUCED, AVERAGE WEIGHT, AND NUMBER OF DAYS REQUIRED TO REACH MATURITY

PERIOD OF TREATMENT	NO. OF SEEDS	NO. GERMINATED	PERCENTAGE GERMINATION	NO. OF MATURE PLANTS	PERCENTAGE MATURED	AV. WEIGHT OF PLANTS (GM.)	DAYS TO REACH MATURITY
Untreated	100	95	95.00	91	91.00	26.26	37
8 hours	120	97	80.83	84	70.00	24.50	38
2 days	120	76	63.33	71	59.17	22.88	38
5 days	140	46	32.86	31	22.14	17.48	43
7 days	140	7	5.00	3	2.14	16.67	48
9 days	140	1	0.71	1	0.71	14.50	46

Figure 2 shows the effect of the seed treatment on growth of the plants. The two plants were at the same stage of development. They were grown in flower pots, and the roots were removed from the soil so as to secure the entire root system. The plant on the left is typical of those grown from untreated seed, that on the right of those grown from seed treated 5 days. Flower buds may be seen on each of them. The plant from untreated seed reached this stage in

44 days, that from treated seed in 50 days. The plants from treated seeds showed marked reduction in growth resulting from the seed treatment. Both the shoot and the root system showed this reduction in growth, apparently in about the same proportion.

FIG. 2.—Effects on subsequent growth of soaking bean seeds in distilled water with aeration: left, plant from untreated seed, 44 days after planting; right, plant from seed soaked with aeration 5 days, 50 days after planting.

In the plants from untreated seeds the average diameter of the internode of the stem immediately above the first pair of leaves was 3.497 mm., the average thickness of the xylem was 0.474 mm., and the average thickness of the phloem was 0.07953 mm. The ratio of the average thickness of the phloem to the xylem was 0.168. In the

plants produced from seeds treated 5 days the average diameter of the same internode of the stem was 3.377 mm., the average thickness of the xylem 0.452 mm., and the average thickness of the phloem 0.1155 mm. The ratio of the phloem to the xylem was 0.255. It will be noted that there is a marked increase in the average thickness of the phloem and in the ratio of the phloem to the xylem in the plants from the treated seeds.

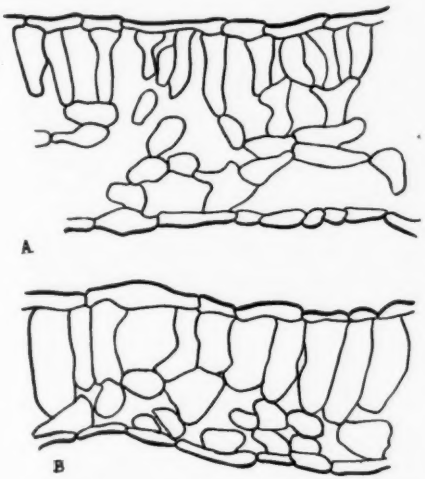

FIG. 3.—Cross-sections of leaves: *A*, section of typical leaf of plant from untreated seed; *B*, section of typical leaf of plant from seed soaked with aeration 5 days.

The difference in leaf structure in the plants from untreated seeds and those from seeds treated 5 days is shown in figure 3. Figure 3 *A* shows the cross-section of a leaf of a mature plant from untreated seed, figure 3 *B* shows that of a mature plant from seed treated 5 days. The sections shown in the figure are representative of the leaf structure found generally in the two sets of plants, the sections being drawn to the same scale. There is a marked decrease in thickness and increase in compactness of structure in the leaf of the plant from the treated seed.

EXPERIMENT II.—This experiment deals with the influence of soaking seeds in distilled water with aeration on germination, num-

ber of mature plants produced, average weight, time required to reach maturity, percentage of dry matter and water content, abundance of carbohydrates and nitrogenous compounds; and the influence on growth of the presence of a calcium salt in very dilute solution in the water in which the seeds were soaked.

Because of the shorter days and lower light intensity of late autumn and winter, there was but little branching in these plants, and their size and weight were much less than in the plants in experiment I. The plants grew and matured normally, however, and the results

TABLE II

GERMINATION OF SEEDS (APPEARANCE AT SURFACE OF SOIL), NUMBER OF MATURE PLANTS PRODUCED, AVERAGE WEIGHT, AND NUMBER OF DAYS REQUIRED TO REACH MATURITY:

1, SEEDS UNTREATED

2, SEEDS SOAKED WITH AERATION 5 DAYS IN DISTILLED WATER

3, SEEDS SOAKED WITH AERATION 5 DAYS IN DISTILLED WATER CONTAINING CALCIUM NITRATE (APPROXIMATELY 900×10^{-6} GM. NORMAL PER LITER)

No.	No. of seeds	No. germinated	Percentage germination	No. of mature plants	Percentage matured	Av. weight of plants (gm.)	Days to reach maturity
1.....	100	90	90.00	80	80.00	2.80	41
2.....	140	98	70.00	54	38.57	1.86	48
3.....	105	71	67.62	48	45.71	2.08	48

indicate similar effects from seed soaking in reduction of growth. There is a decrease in germination and a greater decrease in the number of plants able to reach maturity, a marked reduction in average weight, and an increase in the time required to reach maturity. The results obtained from the addition of calcium nitrate to the water indicate that in general this addition did not greatly modify the effects of the soaking. The results are shown in tables II and III.

The data given in table III indicate that there is an increase in the percentage of dry matter in the plants produced from seeds soaked with aeration 5 days in distilled water, in comparison with the plants from untreated seeds.

One of the most striking results of the predetermining influence of soaking the seeds is the relative increase in the carbohydrates in

the plants produced from the treated seeds, in comparison with those from untreated seeds. This increase is seen in the reducing

TABLE III

PERCENTAGE OF DRY MATTER AND MOISTURE

SEED TREATMENT	PERCENTAGE	
	DRY MATTER	MOISTURE
Untreated	16.61	83.39
5 days in H_2O	19.05	80.95

TABLE IV

GLUCOSE, SUCROSE, AND TOTAL SUGARS EXPRESSED AS PERCENTAGES ON TOTAL-WEIGHT AND DRY-WEIGHT BASES

SEED TREATMENT	WEIGHT BASIS					
	GLUCOSE		SUCROSE		TOTAL SUGARS	
	TOTAL	DRY	TOTAL	DRY	TOTAL	DRY
Untreated	0.299	1.800	0.309	1.921	0.608	3.721
5 days in H_2O	0.443	2.320	0.344	1.804	0.787	4.124

TABLE V

STARCH, HEMICELLULOSES, AND TOTAL POLYSACCHARIDES EXPRESSED AS PERCENTAGES ON TOTAL-WEIGHT AND DRY-WEIGHT BASES

SEED TREATMENT	WEIGHT BASIS					
	STARCH		HEMICELLULOSES		TOTAL POLYSACCHARIDES	
	TOTAL	DRY	TOTAL	DRY	TOTAL	DRY
Untreated	0.672	3.857	1.212	7.481	1.883	11.338
5 days in H_2O	1.635	8.587	1.330	6.985	2.965	15.572

sugars and total sugars, starch, and total polysaccharides. These results are shown in tables IV–VII. The reducing sugars are computed as glucose and the non-reducing sugars as sucrose.

The data given in tables VIII and IX indicate that the predetermining influence of soaking the seeds resulted in a decrease in total nitrogen, organic nitrogen, amino-acid nitrogen, and proteins.

TABLE VI

TOTAL CARBOHYDRATES EXPRESSED AS PERCENTAGES ON TOTAL-WEIGHT AND DRY-WEIGHT BASES

SEED TREATMENT	WEIGHT BASIS	
	TOTAL	DRY
Untreated	2.491	15.059
5 days in H_2O	3.752	19.699

TABLE VII

CARBOHYDRATES EXPRESSED AS PERCENTAGES OF WEIGHTS OF THE CARBOHYDRATES FOUND IN PLANTS FROM UNTREATED SEEDS; BASIS OF EQUAL WEIGHTS OF WET AND DRY MATTER OF SAMPLES

SEED TREATMENT	WEIGHT BASIS											
	GLUCOSE		SUCROSE		TOTAL SUGARS		STARCH		HEMICELLULOSES		TOTAL CARBOHYDRATES	
	WET	DRY	WET	DRY	WET	DRY	WET	DRY	WET	DRY	WET	DRY
None	100	100	100	100	100	100	100	100	100	100	100	100
5 days in H_2O	148	129	111	94	129	111	243	223	110	93	151	131

TABLE VIII

TOTAL NITROGEN, ORGANIC NITROGEN, AMINO-ACID NITROGEN, AND PROTEINS EXPRESSED AS PERCENTAGES OF WEIGHTS OF WET AND DRY MATTER OF SAMPLES

SEED TREATMENT	WEIGHT BASIS							
	TOTAL NITROGEN		ORGANIC NITROGEN		AMINO-ACID NITROGEN		PROTEINS	
	WET	DRY	WET	DRY	WET	DRY	WET	DRY
None	0.623	3.749	0.619	3.729	0.021	0.126	2.745	16.531
5 days in H_2O	0.543	2.851	0.540	2.836	0.017	0.088	2.545	13.363

EXPERIMENT III.—This experiment, designed to measure the effects of seed soaking upon subsequent respiration, presents a quantitative determination of the carbon dioxide given off in respiration by treated and untreated seeds after sprouting. As is shown by table X, there was no decrease in respiratory carbon dioxide from sprouted seeds in the case of the soaked seeds in comparison with those not soaked. On the contrary, the results showed an increase.

TABLE IX

TOTAL NITROGEN, ORGANIC NITROGEN, AMINO-ACID NITROGEN, AND PROTEINS EXPRESSED AS PERCENTAGES OF WEIGHTS OF THESE FOUND IN PLANTS FROM UNTREATED SEEDS; BASIS OF EQUAL WEIGHTS OF WET AND DRY MATTER OF SAMPLES

SEED TREATMENT	WEIGHT BASIS							
	TOTAL NITROGEN		ORGANIC NITROGEN		AMINO-ACID NITROGEN		PROTEINS	
	WET	DRY	WET	DRY	WET	DRY	WET	DRY
None	100	100	100	100	100	100	100	100
5 days in H_2O	87	76	87	76	80	70	93	81

TABLE X

WEIGHT OF RESPIRATORY CARBON DIOXIDE GIVEN OFF PER 100 GM. OF DRY MATTER IN 10 HOURS

	SEED TREATMENT	
	UNTREATED	3 DAYS IN H_2O
Grams of CO_2	1.191	1.568

EXPERIMENT IV.—It was thought that soaking the seeds might influence their catalase activity. The data given in table XI indicate that in the case of seeds soaked with aeration there is a small decrease in catalase activity in the seeds soaked 2 days, but an increase in those soaked 5 days. On the other hand, table XII shows that in the case of the seeds soaked without aeration there is a considerable decrease in catalase activity resulting from soaking for 2 days, and a further decrease from a soaking period of 5 days. The

volume of oxygen has been computed for 1 gm. of air-dry material and 1 gm. of dry matter.

TABLE XI

VOLUME OF OXYGEN LIBERATED BY CATALASE ACTION AND PERCENTAGE OF OXYGEN ON BASIS OF THAT LIBERATED BY UNTREATED SEEDS; SEEDS SOAKED WITH AERATION; VOLUMES CORRECTED TO 0° C. AND 760 MM. PRESSURE; 10-MINUTE RUNS

SEED TREATMENT	0.2 GM AIR-DRY MATERIAL (CC.)	1.0 GM AIR-DRY MATERIAL (CC.)	1.0 GM. DRY MATTER (CC.)	PERCENTAGE OXYGEN
Untreated.....	19.97	99.85	113.04	100.00
2 days in H_2O...	19.04	95.20	108.02	95.56
5 days in H_2O...	22.49	112.45	129.47	114.53

TABLE XII

VOLUME OF OXYGEN LIBERATED BY CATALASE ACTION AND PERCENTAGE OXYGEN ON BASIS OF THAT LIBERATED BY UNTREATED SEEDS; SEEDS SOAKED WITHOUT AERATION; VOLUMES CORRECTED TO 0° C. AND 760 MM. PRESSURE; 10-MINUTE RUNS

SEED TREATMENT	0.2 GM. AIR-DRY MATERIAL (CC.)	1.0 GM. AIR-DRY MATERIAL (CC.)	1.0 GM. DRY MATTER (CC.)	PERCENTAGE OXYGEN
Untreated.....	23.02	115.10	125.89	100.00
2 days in H_2O...	20.32	101.60	111.87	88.86
5 days in H_2O...	19.27	96.35	106.43	84.54

Discussion

KIDD and WEST (**4**) found no decrease in the rate of germination in bean seeds soaked 6 hours, and but little decrease from a treatment of 24 hours; but practically complete failure of germination resulting from treatment for 72 hours. Their report shows no provision for aeration of the seeds or elimination of bacteria. TILFORD, ABLE, and HIBBARD (**19**), who sterilized the seeds to eliminate bacteria and aerated them during the soaking treatment, came to the conclusion that bean seeds may be expected to germinate as well under water as in the soil, provided there are sufficient oxygen for respiration, removal of the carbon dioxide and other by-products, and elimination of the bacteria. RHINE (**15**) found that wheat soaked in sterilized distilled water 22 days gave a germination test of only 2

per cent, while that soaked 29 days showed a complete failure of germination. BARTON (1) concluded that "the capacity for germination is not destroyed by long continued soaking in sterile media."

In this investigation, the seeds after soaking were planted at a depth of approximately 1 inch in the soil. Germination was considered to have taken place when the seedlings appeared at the surface of the soil. The results of the germination tests are shown in table I. It will be noted that soaking for 8 hours decreased the rate of germination from 95 to 80.83 per cent, and that there was a progressive decrease in the rate of germination as the length of the period of seed treatment was increased. The rate of germination was reduced to 5 per cent by 7 days' treatment. It should be borne in mind that in these experiments the seeds were freed from bacteria by sterilization, and that they were aerated during the period of soaking. The seeds were subjected to the action of a large volume of water, since the water used in soaking was completely changed four times per day. It is evident that soaking in distilled water had an injurious effect on germination, and that germination practically ceased when the seeds had been subjected to soaking for 7–9 days. These results seem to differ radically from those obtained by KIDD and WEST, TILFORD, ABLE, and HIBBARD, and BARTON. In the work of these investigators, the seeds were subjected to the action of comparatively small volumes of water, since the water was not changed during the soaking period. Also these workers did not germinate the seeds in the soil, while in this investigation the seeds were planted in the soil and the seedlings had to grow sufficiently to appear at the surface of the soil before the seeds were considered to have germinated. Table II shows the injurious effect on germination of a prolonged period of soaking. Data given in tables I and II show the further reduction in the number of plants from soaked seeds which were able to reach maturity, a considerable number of plants from treated seed dying before the mature stage was reached.

The plants photographed in figure 2 show the deleterious influence of seed soaking on subsequent growth of the plant. The reduction in growth in the shoot and root system is in about the same proportion. The increase in time required for the plants from treated seeds to reach the blooming stage also indicates the injurious effect of the

seed treatment on the rate of growth. This is further shown in the progressive decrease in the average weight of the plants (tables I, II).

TRUE (**20**) studied the harmful action of distilled water and the significance of calcium for higher green plants, using the electrical conductivity method. He found that the samples of distilled water showing the highest electrical resistance were in general more harmful to lupine roots than water containing larger quantities of electrolytes. He also found that these samples of water withdrew electrolytes from the tissues of the roots. This was taken to be the probable mechanism causing the harmful action. According to TRUE, the distilled water seems to withdraw material required for maintenance of the efficient action of the protoplasmic limiting membranes, with the result that cell permeability is increased and a further dissociation of the electrolytes from their points of combination in the proteins and other chemical structures of the cells ensues; but when a calcium salt is added to the distilled water in sufficient concentration to make it osmotically equal to tap water, this dissociating power of the water is largely undeveloped, the leaching is checked, and the chemical integrity of the cells is maintained. TRUE and BARTLETT (**21**) came to the conclusion that there are equilibrium concentrations for calcium and nitrate ions and for magnesium and nitrate ions at which leaching is largely overcome, and below which roots would leach ions into either solution or into both solutions mixed in various proportions. It was shown that calcium ions differ from magnesium ions in being harmless in concentrations that prove fatal in the case of magnesium. In no concentration of a calcium salt that was tried, the strongest being approximately 900×10^{-6} gm. normal per liter, was there any indication of injury.

SCARTH (**16**) investigated the toxic action of distilled water on *Spirogyra* and its antagonism by cations. He came to the conclusion that distilled water free from metal ions but exposed to the air is highly toxic to this plant, that the positive toxic factor is the H-ion concentration due to the dissolved CO_2, and that cations are antagonistic to this toxic action according to their valencies, being limited also by their own toxicity.

In this investigation, experiment II was modified to determine

whether the addition of a calcium salt to the distilled water in which the seeds were soaked would decrease or modify the deleterious influence of soaking on subsequent growth of the plants. The highest concentration of calcium nitrate employed by TRUE, approximately 900×10^{-6} gm. normal per liter, was used. The data in table II lead to the conclusion that the injurious effects of soaking on the rate of germination and on subsequent growth were not greatly modified by the addition of the calcium salt. Table III shows that one of the results of soaking is an increase of dry matter or decrease in moisture in the mature plants produced, in comparison with plants produced from untreated seeds.

One of the very evident effects of the predetermining influence of seed soaking is the higher percentage of carbohydrates in the mature plants from the treated seeds. This is shown in tables IV–VII. The percentage of reducing sugar is higher in the plants from the treated seeds, and the increase in starch resulting from the seed treatment is very striking.

The data in tables VIII and IX lead to the conclusion that another effect of the predetermining influence of seed soaking is a relative decrease in total nitrogen, organic nitrogen, amino-acid nitrogen, and proteins. While this decrease in nitrogen compounds is not so pronounced as the increase in carbohydrates, yet it seems to be very evident.

A correlation between the predetermining influence of this seed treatment and the internal metabolism of the plants produced from the treated seeds may be seen in the carbohydrate-nitrogen relations of the plants, and the moisture and dry matter content. The plants from treated seeds, which were higher in carbohydrates and lower in nitrogen and nitrogen compounds, were lower in moisture content and higher in dry matter. On the other hand, the plants from untreated seeds, which were lower in carbohydrates and higher in nitrogen and nitrogen compounds, were higher in moisture and lower in dry matter (**10**).

It seems probable that the greater accumulation of carbohydrates in the plants from the treated seeds is due to a decrease in the ability of these plants to form amino acids, proteins, and related nitrogen compounds from the carbohydrates, resulting from soaking the

seeds. The reduction in growth in these plants may be a result of a reduction in the nitrogen compounds. A comparison of the two plants shown in figure 2 leads to the conclusion that the plant from the treated seed has as extensive a root system in proportion to the shoot as has that from the untreated seed. Evidently the decrease in the nitrogen compounds and water in the plants from the treated seeds is not due to reduced absorption caused by a poorly developed root system.

The data in table X indicate that there was no decrease in the production of respiratory carbon dioxide by the sprouted seeds resulting from soaking. Some of the soaked seeds did not sprout, of course, and only sprouted seeds were used in the test. This result indicates that the soaking treatment does not injure the respiratory enzymes or mechanism of the seeds, and that the reduction in growth is not due to a decrease in the rate of respiration. In fact, the production of respiratory carbon dioxide was somewhat higher in the sprouted seeds previously soaked than in those not previously soaked. Probably the higher water content of the former was more favorable to the respiratory process. The water content of the sprouted seeds previously soaked, at the stage at which the test for respiratory carbon dioxide was made, was 63.9 per cent; while that of the seeds sprouted without being previously soaked, at the same stage, was 53.7 per cent. MAXIMOV (11) gives the results obtained by KOLKWITZ in connection with the relation of moisture to respiration in barley. He found that 1 kg. of barley grain containing 10–12 per cent of moisture exhaled in 24 hours 0.0003—0.0004 gm. of carbon dioxide; with 14–15 per cent moisture, 0.0013–0.0015 gm. of carbon dioxide; and that with a further increase in water content, respiration increased rapidly, reaching 2.0 gm. of carbon dioxide in 24 hours, when the grain was almost completely swollen.

Table XI indicates that soaking 2 days in distilled water with aeration caused a decrease of 4.44 per cent in the oxygen liberated from hydrogen peroxide by the catalase activity of the seeds, while soaking with aeration 5 days caused an increase of 14.53 per cent. On the other hand, the data in table XII show that soaking without aeration resulted in a continuous decrease in catalase action, amounting to 11.14 per cent in the seeds soaked 2 days and 15.46 per cent in

those soaked 5 days. The latter result is in harmony with that obtained by RHINE (15). In using wheat soaked (without aeration) in distilled water previously boiled and cooled, she found that there was a continuous decrease in catalase activity resulting from soaking, the catalase action practically ceasing after a soaking period of 29 days. In the case of soaking with aeration, the seeds passed through the early stages of germination during the soaking treatment. There was the usual development of catalase activity in connection with germination, amounting to an increase of 14.53 per cent when the seeds had been soaked 5 days.

Summary

1. Soaking seeds of dwarf bean in distilled water with aeration resulted in a considerable decrease in the rate of germination (appearance of the seedlings at the surface of the soil) for periods of soaking as short as 8 hours. Practically a complete failure of germination resulted from soaking periods of 7 to 9 days.

2. The soaking treatment resulted in a still further reduction in the number of mature plants produced, as some of the seedlings from the treated seeds were unable to reach maturity.

3. The soaking treatment resulted in a progressive decrease in growth and weight of the plants produced and an increase in the time required for the plants to reach maturity.

4. The seed treatment resulted in general in a modification of the leaf structure, the leaf blades of plants from treated seeds generally being thinner and more compact in structure than those of plants from untreated seeds.

5. The greater average thickness of the phloem and higher ratio of the phloem to the xylem in the stems of the plants from soaked seeds is probably correlated with the relatively higher carbohydrate and lower nitrogen content of these plants.

6. The addition of calcium nitrate in very dilute solution to the water in which the seeds were soaked did not prevent the deleterious influence of soaking on germination and growth.

7. Soaking the seeds resulted in a marked relative increase in reducing sugars, starch and total carbohydrates, and solid matter, and in a relative decrease in total nitrogen, organic nitrogen, amino-acid

nitrogen, and proteins in the mature plants produced from the soaked seeds. A correlation may be seen in the decreased nitrogen associated with decreased moisture and increased carbohydrates.

8. It seems probable that the greater relative accumulation of carbohydrates and deficiency of nitrogen compounds in the plants from the soaked seeds are due to a decrease in the ability of these plants to form the nitrogen compounds from the carbohydrates. The reduction in growth is probably due to the reduction in nitrogen compounds.

9. There was an increase in the rate of production of respiratory carbon dioxide in the sprouted seeds soaked before sprouting in comparison with those sprouted without previous soaking. This increase was probably due to the fact that the treated seeds contained considerably more water than untreated ones. The higher water content was favorable to a more rapid rate of respiration. The reduction in growth, of course, was not connected with any decrease in the rate of respiration.

10. Soaking the seeds without aeration resulted in a regular decrease in the catalase activity. Soaking with aeration resulted at first in a small decrease in catalase activity, but this was soon followed by a considerable increase. This modification of the result is evidently due to the fact that the seeds when soaked with aeration pass through the early stages of germination, and consequently there is an increase in catalase activity characteristic of seed germination.

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PARALLELISM OF PRECIPITATION REACTIONS AND BREEDING RESULTS IN THE GENUS IRIS[1]

I. PRELIMINARY STUDY AND CORRELATION WITH OTHER EVIDENCE

ROBERT C. FOSTER AND GEORGE S. AVERY, JR.

(WITH ONE FIGURE)

Introduction

A preliminary study has been made of the precipitation reactions between some of the more distinct subgroups of the genus *Iris*. The purpose has been to determine whether a correlation exists between the taxonomy of the genus and the precipitation reactions of its species. Evidence from breeding experiments, from the few known chromosome numbers, and from chemical analyses is discussed also in this connection. Further investigations will include other groups of the genus. By placing the chief emphasis on the reactions within one genus, this study differs somewhat from the work of KOSTOFF (14) and CHESTER (5).

The classification of *Iris* which is generally followed is that proposed by DYKES (8). It is used, with a few changes in order, by DIELS in his treatment of Iridaceae, in ENGLER and PRANTL (10). As a result of their recent discoveries in Louisiana, SMALL and

[1] At the suggestion of Professor G. K. K. LINK, we have used "precipitation reaction" in place of "normal precipitin reaction," as proposed by others. We quote from his letter:

> "*Precipitin reaction* is a term used in serology and immunology for a specific type of precipitation reaction. It involves the reaction of the blood sera of animals that have been modified *in vivo* by the presence of foreign substances that contain or consist of proteins or protein combinations with other substances. These substances, whether particulate or in solution, are designated as antigens, because they engender specific antibodies in blood sera. If the presence of such antibodies is detectable *in vitro* by a reaction involving precipitation of a combination of the antibody and of a test antigen which is in solution, the antibody is designated as a *precipitin* and the reaction a *precipitin reaction*. Antigens may get into the blood stream spontaneously, or by artificial means, such as injection. Precipitins engendered by anti-

ALEXANDER (**21**) have adopted a new partial classification, one, however, which they themselves term "incomplete and necessarily tentative."

The approximately 160 species of *Iris* recognized by DYKES are placed in two major groups characterized by different habit, the rhizomatous and the non-rhizomatous. Rhizomatous irises fall into seven sections, and non-rhizomatous (bulbous) into three sections, marked not only by taxonomic differences but also by differences in geographic distribution. In addition, there are two other small sections, one of two species, characterized neither by bulb nor rhizome, said by DYKES (**9**) to resemble *Hemerocallis*, and one further species, *Iris sisyrinchium* L., which has a corm and is more nearly related to *Moraea* than to *Iris* (**9**).

Of the 12 sections of *Iris*, the most wide-spread is the Apogon (beardless) section, members of which are found in Europe, North Africa, and America. This great range is accompanied by marked diversity of habitat, unlike other sections of the genus. It is in this group also that there is found the greatest range of chromosome numbers, from n = 12, in *Iris pseudacorus* L. and others, to n = ca. 56 in *I. virginica* Dickson (**11, 12**). The large section Pogoniris (bearded) is confined to a smaller range throughout Europe, North Africa, and the Near and Middle East. Its members display marked

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gens not artificially introduced are called *normal precipitins*, and the test reaction a *normal precipitin reaction*.

The plant reactions reported by KOSTOFF, CHESTER, and in your paper consequently are neither normal precipitin reactions nor precipitin reactions following injections of antigens. Unfortunately, the latter have been referred to as "acquired precipitin reactions" in the botanical literature.

Obviously, in order to avoid confusion and misunderstanding, until more is known about the nature of the reactions involved in the direct tests between plant extracts and in the tests using blood antisera, it is advisable to avoid the serological and immunological terms which now have definite meanings. I had decided before receipt of your paper to suggest in a brief paper that the non-committal term "precipitation reaction" be used for the interesting reactions of plant extracts which do not involve blood sera, until it is demonstrated whether these and the precipitin reactions are fundamentally identical or dissimilar."

While the precipitin test is one involving antigens and antibodies engendered in blood sera by antigens, the precipitation reaction as referred to in this paper simply indicates the formation of precipitates in which antigens and blood sera are not involved.

uniformity in habitat, and the known chromosome numbers fall into distinct groups, correlated with the taxonomic subdivisions, varying as follows: n = 12, n = 20, n = 22, and n = 24 (**11, 12**). The Oncocyclus, Regelia, and Pseudoregelia sections are confined to even smaller ranges in the Near East and Central Asia. They likewise display great uniformity in chromosome numbers; in Oncocyclus 2n = 20, and in Regelia 2n = 44, with few exceptions (**11, 12**). Each of the three bulbous sections, Juno, Reticulata, and Xiphium, is found in a relatively small geographic range; with but one exception all the species require a warm, dry habitat; the few known chromosome numbers range from n = 9 in *I. caucasica* Hoff. to n = 21 in *I. xiphioides* Ehrh. (**11, 12**). The small Evansia (crested) section has representatives only in southeastern Asia and the United States, and the chromosome numbers vary from n = 12 in *I. cristata* Ait. to n = 18 in *I. japonica* Thunb. (**11, 12**). It might be noted that SMALL and ALEXANDER (**21**) remove *I. verna* L. and *I. cristata* to another genus, *Neubeckia* Alef.

Material and methods

The species, varieties, and hybrids used in this study represent six of the twelve sections of the genus, and include eight of the fifteen subsections of the Apogon section. They were secured from the *Iris* collection at Connecticut College and from Mrs. LOUISE W. KELLOGG of West Hartford, Connecticut, who has generously provided us with material on two occasions. Wherever possible, only species and varieties were used, but certain horticultural hybrids of known parentage have been included, as well as a beardless Japanese iris of unknown parentage. So far as is known, all material was correctly identified; but the possibility of misidentification must not be left out of consideration, especially as most of the material was collected after the blooming period was over. The following plants were used: a recognized albino form of *I. orientalis* Thunb., called Snow Queen, *I. forrestii* Dykes, *I. prismatica* Pursh, *I. unguicularis* Poir., *I. graminea* L., *I. halophila* Pall., *I. ochroleuca* L., a probable descendant of *I. kaempferi* Sieb. called Albatross, *I. pseudacorus* L., *I. pseudacorus* var. *bastardi* Spach., *I. fulva* Ker-Gawl., *I. hexagona* Walt., *I. foliosa* Mackenzie and Bush, two hybrids of *fulva*×*foliosa* parentage called Fulvala and Dorothea K. Williamson, *I. chryso-*

phoenicia Small, *I. hyacinthiana* Farrer, *I. tricuspis* Dickson (probably merely a variety of one of the Tripetalae), *I. setosa* Pall. var. *canadensis* Foster, *I. verna* L., *I. vinicolor* Small, *I. dichotoma* Pall., *I. tectorum* Maxim., *I. gracilipes* Gray, *I. cristata* Ait., *I. stolonifera* Maxim. var. *vaga* Foster, *I. hoogiana* Dykes, *I. kochii* Kerner, Marjory Tinley (a garden hybrid between two forms of *I. pallida* Lam.), and *I. bucharica* Foster.

In general, the technique used in this study was that of KOSTOFF (**14**) and CHESTER (**5**), but certain modifications were found necessary. Leaves of the plants to be studied were collected, weighed, and washed in tap and distilled water. After being cut into pieces as small as possible, they were ground in unglazed mortars; the paste thus obtained was placed in an Erlenmeyer flask and to it was added distilled water to the amount of twice the weight of the green leaves. The flasks were then placed in a cold chamber at 3°–5° C. After 24 hours the extracts were filtered into test-tubes (one filtering usually being sufficient) and again placed in the cold chamber. Some were covered with a thick layer of toluene for preservation over a longer period of time. The extracts were mostly crystal clear and colorless, although some had a slight yellowish tinge, and that of *I. pseudacorus* was opalescent.

An attempt to use extracts from dried material as advocated by CHESTER (**5**) proved unsatisfactory, since no results were obtained from such extracts against each other; on the other hand, in some instances these extracts from dried material gave positive reactions when tested against extracts from green material.

In making the tests, small tubes of several sizes were used, varying from 3 to 8 mm. in diameter and 50–70 mm. in length. It was found that in most cases a 6-mm. tube gave the best results. The heavier liquid was placed in a tube, and the lighter liquid carefully placed above it by means of a fine pipette drawn out to a tip opening approximately 0.5 mm. in diameter. Observations were then made over a period of 45 minutes. In a positive reaction, a ring of precipitate of varying intensity was formed between the two liquids. This ring gradually widened until, in some instances, both liquids became cloudy. KOSTOFF (**14**, p. 47) noted that in many cases where precipitation was very weak, the precipitate completely disappeared after a time.

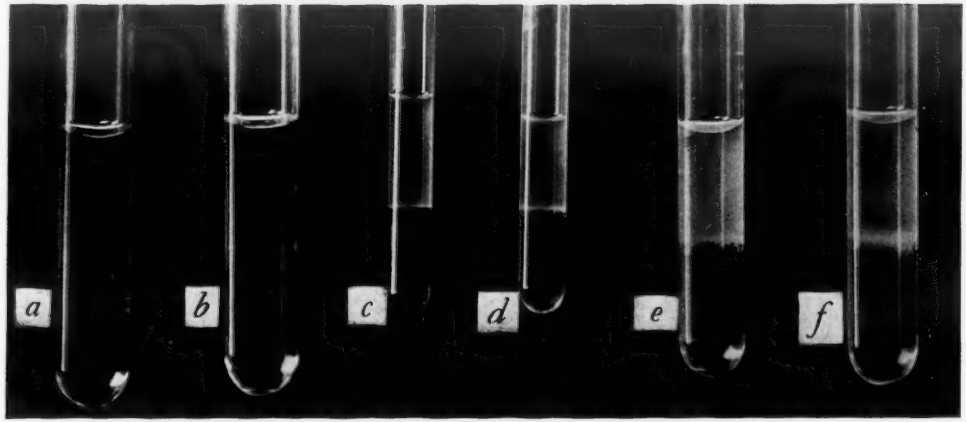

FIG. 1.—*a*, precipitation test between extracts of *I. fulva* and *I. orientalis*, showing no reaction (−); *b*, similar test between extracts of *I. foliosa* and *I. orientalis*, showing a trace (±); *c*, similar test between extracts of *I. fulva* and *I. pseudacorus*, showing a (+) reaction; *d*, similar test between extracts of *I. tectorum* and *I. pseudacorus*, showing a (++) reaction; *e*, *f*, later stages of *c* and *d* respectively.

No instance of such a disappearance was noted in any of our tests, although tubes were sometimes allowed to remain for a long period. He noted (**15,** p. 99) also that different individuals of the same species may give reactions of differing intensities. In some cases our tests confirm this point. No lytic rings appeared. The results of the tests are given in table I, all readings being minimal, and blank spaces in the table indicating that no test was made. The small amount of material available in some cases made a complete series of tests impossible.

To prevent any contamination, mortars, pestles, flasks, large tubes, and glass funnels were cleansed in a concentrated sulphuric acid-potassium bichromate solution overnight, followed by thorough washing in tap and distilled water. Small tubes and other instruments were boiled for 2 hours in a 0.5 per cent solution of sodium carbonate, followed by 4 hours of boiling in distilled water changed four times.

Discussion

Because of the difference in the significance of a positive (+) reading in the precipitation reaction (the "normal precipitin reaction" of KOSTOFF and CHESTER) and the true precipitin reactions, a brief explanation will make the following discussion clearer, particularly so because the precipitin reactions are of long standing in the field of immunology and are therefore better understood.

In the precipitation reaction, as CHESTER (**5**) has pointed out, an increasing positive (+) reaction between extracts of different species seems to indicate an increasing degree of divergence between such species, while the negative (−) reaction may indicate either very close relationship or great divergence. This is in contrast to the precipitin reaction (involving previous injection of an extract into an animal body and subsequent building up of an immunity to that extract), where an increasing (+) reaction is reported to indicate increasingly closer relationship (MEZ and others), the (−) reaction indicating only divergence.

Since the use of extracts from dried material gave unsatisfactory results in these tests, their reactions, especially negative ones, must be regarded as questionable. It will be necessary to retest the species so used with extracts from green material. Furthermore, the study

includes only half the sections, slightly more than half the subsections of the largest section, and less than one-fifth of the recognized species of the genus. In view of these things, any conclusions that are drawn must be regarded as tentative.

Among the Sibericae, it will be noted that *I. prismatica* reacted more strongly and more frequently than the other two Sibericae studied. DYKES (**9**, p. 101) has pointed out certain morphological differences that separate *I. clarkei* Baker and *I. prismatica* from the rest of the Sibericae, and has suggested that, in the case of the former at least, these differences are almost great enough to justify the erection of a separate subsection. The more pronounced precipitation reactions of *I. prismatica* seem to support DYKES' suggestion (table I), and still further support is found in the breeding record of *I. prismatica* (**4, 13, 17**). Crossed with 16 species and varieties with varying success, it has apparently not been crossed successfully with members of the Sibericae, although it has produced seed in crosses with *I. pseudacorus* (Laevigatae) and *I. foliosa* (Hexagonae).

The Laevigatae are outstanding in their reactions. There are positive reactions of varying intensities between them and all other sections and subsections studied, except Vernae (table I). With the Sibericae, however, there is but one positive reaction as compared to ten negative reactions. The preponderance of negative reactions between these two groups might indicate either closeness or divergence of relationship, a decision being facilitated by consideration of the breeding evidence (**3, 4, 7, 13, 17**). Not including the Sibericae, 125 crosses have been recorded between the Laevigatae and all sections and subsections, of which only 18 were successful. Of 94 crosses between the Laevigatae and the Sibericae, 24 were successful. This latter figure becomes even more significant when it is considered that only 18 out of 44 crosses were reported successful between species of the Laevigatae. Clearly the Laevigatae cross more readily with the Sibericae than with other groups, and even these crosses are more successful when the former are used as pollen parents. In this case, the negative precipitation reactions, supported by a good proportion of successful crosses (considering the percentage of successful crosses between members of the group Laevigatae), suggest a comparatively close relationship between the two groups.

TABLE I

PRECIPITATION REACTIONS IN THE GENUS IRIS

d = DRIED MATERIAL; ± = TRACE; + = 1; ++ = 2; − = NO REACTION

SECTION	SUBSECTION	IRIS SPECIES	orientalis (Snow Queen)	forrestii	prismatica	unguicularis	ochroleuca	halophila	graminea	kaempferi (Albatross)	pseudacorus	pseudacorus var. bastardi	hexagona	foliosa	fulva	fulva×foliosa (D. K. W.)	fulva×foliosa (Fulvala)	chrysophoenicia	violipurpurea	hyacinthiana	tricuspis	canadensis	verna	
(I) Apogon†	(1) Sibericae	orientalis (Snow Queen)	− −	−	− −	-				−	+	−	− ±			±	−	±	−	−	−		−	
		forrestii (d)	− −	−	−	±	-			−	−		±		− ±			−	−		−			
		prismatica	− −	+	± ±	-				−	−	−	+ ±		− ±	±		+	+	−			−	
	(8) Unguiculares	unguicularis	− −	+		− −	-			+	+	±	− −		± −	−		−	−				−	
	(9) Spuriae	ochroleuca	− −	±	−		−	-		±	±	±		−	− −	−		−	−			−	−	
		halophila	− ±	±	−	−		±		+	+	±	− −		− ±	* −		−	−	−		−	±	
		graminea	− −	-	−	±				−	−	±	± −		− −	−		−	−					
	(10) Laevigatae	kaempferi (Albatross)	− −	−	+	± ±	-				−	−	++		± +	+		+	+	(++)		++	-	(+
		pseudacorus	+ −	−	+	± +	-			−		−	++		++	(++)	(++)	(++)		+		±	-	(+
		pseudacorus var. bastardi	−	−	±	± ±	±			−	−		± ±		− ±	±		±	−			−		
	(11) Hexagone	hexagona (d)	−	+	−	−	±			+	+	±	−		− −			−	−					
		foliosa	± ±	±	−	− −	−			+	+	±	−		− −	−		−	−			−		
		fulva	− −	−	±	− −	−			±	+	−	− −		−	−		−	−	−		− −	−	
		fulva×foliosa (D. K. Williamson)	− ±	±	−	− ±	* −			+	+	±	− −		−	−		−	−	−		− −	-	
		fulva×foliosa (Fulvala) (d)	−	±	−	− −	−			+	(++)±		−		− −			−	−					
		chrysophoenicia	± −	+	−	− −	−			+	(++)±		− −		− −	−		−	−	−		− −	−	
		violipurpurea	− −	+	−	− −	−			+	(++)−		− −		− −	−		−	−	−		− −	−	
	(12) Ensatae	hyacinthiana (d)	−	−			−			(++)	+				− −			−	−					
	(14) Tripetalae	tricuspis (d)	−				−			+	+	−			− −				−					
		canadensis (d)	−	−	−	− −	−			+	±	−	−		− −			−	−					
	(15) Vernae	verna (d)	−				±			−	−				− −			−	−					
	(unassigned)	vinicolor	− −	+	−	− −	−			(++)	(++)±		− −		− −	−		−	−	−		− −	±	
(II) Pardanthopsis		dichotoma (d)	−	−	−	− −	−			+	+		±		− −			−	−					
(III) Evansia		cristata	− −	−	±	− ±	* ±			+	±	±	− ±		* − −	−		−	−	−		−	-	
		gracilipes (d)	−	±	−	− −	−			−	±				− −			−	−					
		tectorum	− −	−	−	− −	−			+	(++)	−	− −		− −	−		−	−	−		− ±	−	
(V) Regelia		stolonifera var. vaga	− ±	−	−	− −	−			±	(++)	+	− −		− −	−		−	−			−		
		hoogiana (d)	−	−	−	− −	−			+	±				− −			−	−					
(VII) Pogoniris		kochii (d)	−	−		−				(++)	(++)					−		−	−					
		pallida×pallida (M. Tinley)	− −	−	−	− −	−			(++)	+	−	− ±		− −	−		−	−	−		− ±	−	
(IX) Juno		bucharica (d)	−	−	−	− −	−			(++)	±		−		− −			−	−					

* Trace did not appear until after 30 minutes.

† Sections and subsections numbered according to ENGLER and PRANTL.

PRECIPIT

d = DRIED MATERIAL

SUBSECTION		orientalis (Snow Queen)	forrestii	prismatica	unguicularis	ochroleuca	halophila	graminea
ericae	orientalis (Snow Queen)	–	–		–	–	–	–
	forrestii (d)	–	–		–	±		–
	prismatica	–	–		+	±	±	–
guiculares	unguicularis	–	–	+		–	–	–
riae	ochroleuca	–	–	±	–		–	–
	halophila	–	±	±	–	–		±
	graminea	–	–	–	–	–	±	
evigatae	kaempferi (Albatross)	–	–	–	+	±	+	–
	pseudacorus	+	–	–	+	±	+	–
	pseudacorus var. bastardi	–		–	±	±	±	±
kagone	hexagona (d)	–		+	–		–	±
	foliosa	±	±	±	–	–	–	–
	fulva	–	–	–	±	–	–	–
	fulva × foliosa (D. K. Williamson)	–	±	±	–	–	±	* –
	fulva × foliosa (Fulvala) (d)	–		±	–	–	–	–
	chrysophoenicia	±	–	+	–	–	–	–
	violipurpurea	–	–	+	–	–	–	–
satae	hyacinthiana (d)	–		–			–	
petalae	tricuspis (d)	–					–	
	canadensis (d)	–	–	–	–	–	–	–
nae	verna (d)	–					±	
assigned)	vinicolor	–	–	+	–	–	–	–
	dichotoma (d)	–		–	–	–	–	–
	cristata	–	–	–	±	–	±	* –
	gracilipes (d)	–		±	–	–	–	–
	tectorum	–	–	–	–	–	–	–
	stolonifera var. vaga	–	±	–	–	–	–	–
	hoogiana (d)	–		–	–	–	–	–
	kochii (d)	–		–			–	
	pallida × pallida (M. Tinley)	–	–	–	–	–	–	–
	bucharica (d)	–		–	–	–	–	–

r until after 30 minutes.

ions numbered according to ENGLER and PRANTL.

TABLE I

PRECIPITATION REACTIONS IN THE GENUS IRIS

FD MATERIAL; ± = TRACE; + = 1; ++ = 2; − = NO REACTION

IRIS SPECIES

forrestii	prismatica	unguicularis	ochroleuca	halophila	graminea	kaempferi (Albatross)	pseudacorus	pseudacorus var. bastardi	hexagona	foliosa	fulva	fulva × foliosa (D. K. W.)	fulva × foliosa (Fulvala)	chrysophoenicia	violipurpurea	hyacinthiana	tricuspis	canadensis	verna	vinicolor	dichotoma	cristata	gracilipes	tectorum	stolonifera var. vaga	hoogiana	kochii	pallida × pallida (M. Tinley)	bucharica
−	−	−	−	−	−	−	+	−	−±	±	−	−	−	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	±	−	−	−	−	+±	±	−	−±	−	+	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−
−	+	±	±	±	−	−	−	−	+±	±	−	−±	±	+	+	−	−	−	−	+	−	−	±	−	−	−	−	−	−
−+			−	−	−	+	+	±	−−	±	−	−	−	−	−						−	−	±	−	−	−	−	−	−	
−±	−		−	−	−	±	±	±	−	−	−	−	−	−	−						−	−	−	−	−	−	−	−	−	
±±	−		−	±	±	+	+	±	−−	−−	−−	−±*	−	−	−	−	−	−−	±		−	−	±*	−	−	−	−	−	−	
−−	−		−±			−	−	±	±−	−−	−−	−−	−	−	−			−−			−	−	−	−	−	−	−	−	−	
−−	+	±	±	±	−		−	−	++	±+	+	+	+	+	+	(++)	++	−	−	(++)	+	±	−	+	±	+	(++)	(++)	(++)
−−	+	±	±	±	−	−		−	++	++	(++)	(++)	(++)	(++)	(++)	+	++	−	−	(++)	+	±	±	(++)	(++)	±	(++)	+	±
−	±	±	±	±	±	−	−		±±	−±	±	±		±	−		−−			±		±		−	+			−	
+	−		−	−	±	+	+	±	−	−−	−−			−	−		−			−		±	±*	−	−	−	−	−	−	
±±	−		−	−	−	+	+	±	−	−−	−−	−	−	−	−		−			−	−	±	−	−	−	−	−	−	−	
−−	±		−	−	−	±	+	−	−−	−−	−−	−	−	−	−		−			−	−	−	−	−	−	−	−	−	−	
±±	−		−±	*	−	+	+	±	−−	−	−	−	−	−	−		−	−−	−	−	−	−	−	−	−	−	−	−	−	
±	−		−	−	−	+	(++)	±	−	−	−	−	−	−	−		−	−−	−	−	−	−	−	−	−	−	−	−	−	
−+	−		−	−	−	+	(++)	±	−	−	−	−	−	−	−		−	−−	−	−	−	−	−	−	−	−	−	−	−	
−+	−		−	−	−	+	(++)	−	−−	−−	−−	−	−	−	−		−	−−	−	−	−	−	−	−	−	−	−	−	−	
−			−			(++)	+				−	−		−	−					−		−		−						
	−			−		+	+	−			−−			−	−					−		−		−						
−	−	−	−	−	−	+	±	−	−	−	−−			−	−					−	±	−		−	±	−		±	
−+	−	−	−	−	−	(++)	(++)	±	−−	−−	−−	−		−	−	−	−	−	+	−		−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	+	+		±	−−	−−			−	−		−			−		−		−		−		−		
−−	±	±	−±	*	±	+	±	±	−±	*−	−−	−	−	−	−	−	−	−	−	−		−	−	−	±*	−	−	−	−	−
−	±	−	−	−	−	−	±		−	−	−−			−	−		−			−		−		−	−	−		−		
−−	−	−	−	−	−	+	(++)	−	−−	−−	−−	−	−	−	−	−	−	−±	−	−	−		−	−	−	−	−	−	−	−
−±	−	−	−	−	−	±	(++)	+	−−	−−	−−	−	−	−	−		−			−	−	±*	−	−	−	−	−	−	−	−
−	−	−	−	−	−	+	±		−	−	−−			−	−		−			−		−		−		−		−		
−			−			(++)	(++)				−			−	−					−		−		−						
−−	−	−	−	−	−	(++)	+	−	−±	−−	−−	−	−	−	−	−	−	−±	−	−	−		−	−	−	−	−	−	−	−
−	−	−	−	−	−	(++)	±		−	−	−−			−	−					−		−		−		−		−		

PRANTL.

The reactions and breeding results of *I. pseudacorus* (Laevigatae) are more striking than those of any other single species considered here. Of the 11 crosses, reciprocal or otherwise, between *I. pseudacorus* and species, varieties, or hybrids included in this study (table II), eight were unsuccessful. Precipitation reactions between these

TABLE II

CORRELATION BETWEEN BREEDING EVIDENCE AND PRECIPITATION REACTIONS FOR I. PSEUDACORUS AND I. FULVA

♀ PARENT	♂ PARENT	RESULT OF CROSS	RECIPROCAL CROSS	REACTION	AUTHORITY FOR CROSSES
pseudacorus	Snow Queen	S	U*	+	13, 17
pseudacorus	prismatica	U	S	−	4, 13
pseudacorus	graminea	U	U	−	4
pseudacorus	hexagona (d)	U	U*	+	17
pseudacorus	foliosa	U	U*	+	4
pseudacorus	fulva	S	U	+	13, 4
pseudacorus	D. K. W.	U	U	+	17, 4
pseudacorus	tricuspis (d)	U*	U*	+	4
pseudacorus	canadensis (d)	U		±	4
pseudacorus	vinicolor	S		++	13
pseudacorus	tectorum	U		++	13
fulva	Snow Queen	U*	U*	−	13
fulva	pseudacorus	U	S	+	4, 13
fulva	foliosa	S	S	−	4
fulva	D. K. W.	U	S	−	4
fulva	tricuspis (d)	U	U*	−	4
fulva	canadensis (d)	U	U	−	13, 4
fulva	vinicolor	S	S	−	4, 3
fulva	tectorum	U	U	−	13
prismatica	fulva	U*		−	4
graminea	fulva	U		−	4
pseudacorus var. bastardi	fulva	S		−	4
hexagona (d)	fulva	S		−	13
fulvala (d)	fulva	S		−	4
cristata	fulva	U		−	4

Explanation of symbols: (d) indicates use of extract from dried material. In the columns of breeding results, U indicates failure to produce seed; * either parthenocarpy or the production of non-viable seed; and S the formation of viable seed. The reaction symbols are the same as those in table I.

eight different forms and *I. pseudacorus* show one negative, one trace, and six (+) reactions, a rather good correlation with the breeding results. All available records show that *I. pseudacorus*, as a seed parent, has been used successfully in crosses with four other species, unsuccessfully in 22; as a pollen parent it has been used successfully in crosses with nine other species, unsuccessfully in 25. No reciprocal cross is recorded in which it has been successful as

seed and pollen parent. Of these successful crosses with 13 other species, seven were crosses with members of the Sibericae (six if *I. prismatica* is separated from that group) and three were crosses with members of its own subsection, the Laevigatae.

In the crosses between *I. pseudacorus* and *I. orientalis* (Snow Queen), and *I. vinicolor* and *I. fulva* (table II), a new situation arises between the breeding evidence and the precipitation reactions. These species, although crossed with partial success, gave a positive reaction in each case with *I. pseudacorus*. In the case of *I. orientalis*, of six flowers pollinated only one formed a seed capsule, and that one contained few seeds (**13,** p. 24); the reciprocal cross was a failure. From five flowers pollinated in the cross with *I. vinicolor*, only one capsule was formed; it also held few seeds (**13,** p. 24). In the case of *I. fulva*, only five capsules were formed out of 42 pollinations, although each capsule contained many seeds (**13,** p. 24). The positive reaction between such species may well be regarded as an indicator of the relatively low degree of fertility exhibited by such crosses.

No reaction was secured between *I. pseudacorus* and *I. graminea*, and the cross between the species was unsuccessful (**4,** pp. 35, 37). The negative reaction here indicates divergence of the species.

There is often a rather close parallel between the reactions of *I. vinicolor* and species of the group Hexagonae (table I), especially so with *I. chrysophoenicia* and *I. violipurpurea*, suggesting a relationship between them. SMALL and ALEXANDER (**21**) have recognized the relationship between *I. vinicolor* and *I. violipurpurea* by placing them, together with several others, in a new group, the Unicristatae, although they associate *I. chrysophoenicia* with another new group, the Coronicristatae. The preliminary evidence from precipitation reactions would scarcely support the segregating of *I. chrysophoenicia*, but it does support the grouping of *I. vinicolor* with *I. violipurpurea*.

The precipitation reactions and breeding results for *I. fulva* (table II) show cases of close relationship to certain species, and of more distant relationship to others. Of the 14 cases cited, seven show successful crosses, six of which are correlated with negative reactions. In the seven unsuccessful crosses, in each instance there is also a negative reaction. If the negative reaction between some species

indicates divergence (and the evidence thus far in this study partly supports the contention of CHESTER in this regard), the failure of crosses between such species as *I. fulva* and *I. tectorum* is not at variance with the precipitation reaction, but on the contrary supports it. This would hold true of other cases, for example, *I. cristata* ×*I. fulva*, *I. tectorum*×*I. vinicolor*, *I. prismatica*×*I. tectorum*, etc.

TABLE III

CORRELATION BETWEEN BREEDING EVIDENCE AND PRECIPITATION REACTIONS FOR I. FOLIOSA (D. K. WILLIAMSON), I. GRAMINEA, AND I. PRISMATICA

♀ PARENT	♂ PARENT	RESULT OF CROSS	RECIPROCAL CROSS	REACTION	AUTHORITY FOR CROSSES
foliosa	Snow Queen	U		±	13
foliosa	pseudacorus	U*	U	+	4
foliosa	D. K. W.	S	S	−	4
foliosa	fulva	S	S	−	4
foliosa	vinicolor	U*	U	−	13, 4
prismatica	foliosa	S		±	4
D. K. W.	prismatica	U		±	17
D. K. W.	pseudacorus	U	U	+	4, 17
D. K. W.	foliosa	S	S	−	4
D. K. W.	fulva	S	U	−	4
vinicolor	D. K. W.	S		−	4
graminea	pseudacorus	U	U	−	4
graminea	fulva	U		−	4
halophila	graminea	U		±	4
prismatica	Snow Queen	U*		−	13
prismatica	pseudacorus	S	U	−	13, 4
prismatica	foliosa	S		±	4
prismatica	fulva	U*		−	4
prismatica	vinicolor	U*		+	4
prismatica	tectorum	U*		−	13
D. K. W.	prismatica	U		±	17

Symbols are the same as in table II.

Where the morphological differences are great, as in the cases cited, the negative reaction ought almost certainly to be interpreted as indicating divergence.

Table III gives a similar comparison for some of the other irises studied. *I. foliosa* and D. K. Williamson (*I. fulva*×*I. foliosa*) show a good correlation, while *I. prismatica* and *I. graminea* do not. The last-named species presents certain peculiarities which help to explain such behavior. Table I shows that the reaction between *I.*

graminea and *I. halophila* is the only instance of a reaction between members of the same subsection. Moreover, the chromosome number of *I. graminea* is reported as n = 17 (**11**), differing markedly from other members of the group. In *I. spuria* L., n = 12 (**11**), in *I. ochroleuca*, n = 20 (**11**), and in *I. halophila*, n = 22 (**11**). These four are the only species of the subsection whose chromosome numbers are known, but the reported presence of the uneven number in *I. graminea* can hardly be without significance. No cross utilizing this species is reported as having been successful, although 16 species and varieties were used in the work (**4**). Even within its own subsection it has not been crossed successfully. The negative breeding results, combined with the generally negative precipitation reactions, are strongly suggestive of a rather solitary position for *I. graminea*.

It was the behavior of embryos in species crosses in *Nicotiana* that suggested to KOSTOFF (**15**, p. 35) that "immunological phenomena are involved in hybridization and during the ontogeny of hybrid embryos." In regard to *Linum*, LAIBACH (**16**) has pointed out that the death of embryos in seeds must not be regarded as proof of genetic lethality, since the seed parent may not be qualified to afford normal development to otherwise viable seeds. He states: "Because embryo and mother-plant are mutually adapted in their ontogeny, disturbances in the physiological relations can be produced by genotypical differences that are the reason for the death of the embryo." He reports demonstrating this by removing the hybrid embryos of a cross which does not produce viable seeds, and maturing them successfully on cotton in a sugar solution, growing plants from some of the seeds thus matured. This lends strength to the view that in some instances, certainly, the failure to produce seed is due to the hostile environment in which the embryos are developing.

Similar cases apparently exist in species crosses in *Iris*. Sometimes there is parthenocarpy, and sometimes dead embryos can be found in the seed capsules, for example, in the cross *I. pseudacorus* × *I. versicolor* (**20**). The reciprocal cross, *I. versicolor* × *I. pseudacorus*, will usually produce a few seeds (**4**, p. 41); and indeed, like the Laevigatae in general, *I. pseudacorus* seems rather more successful as a pollen parent than as a seed parent. This failure to produce

viable seed in one direction in a reciprocal cross might be attributed in this case to "endosperm deficiency," as has been done in other similar cases by THOMPSON (**22**), who suggests that in reciprocal interspecific crosses which differ in the success with which they can be made, the more successful of the pair is that in which the species with the higher chromosome number is used as the seed parent. Since the chromosome number of *I. pseudacorus* is n = 12 (**11**), and the chromosome number of *I. versicolor* is reported as ranging from ca. 36 to ca. 56 (**11**), this explanation seems to apply to the case in question. On the other hand, it seems more reasonable to suppose that this endosperm deficiency is perhaps not the *causa causans* of the death of the embryos, but is itself simply an expression of physiological or immunological incompatibility, as suggested by LAIBACH and KOSTOFF.

The "immunological" interpretation of the precipitation reaction is discouraged by CHESTER (**6**), who has found strong evidence in certain woody plants against the theory that the reaction is of a protein nature. He reports that nearly 57 per cent of the reactions observed in his work consisted of calcium oxalate precipitations, and states (**6,** p. 295): "This precipitation of calcium oxalate is so frequently the sole or main phenomenon in the reactions considered immunological in nature as to invalidate immunological interpretations laid on such reactions in which the calcium oxalate factor is not removed." The remaining factors he interprets by assuming the presence or absence of three other pairs of reactive substances. Although this tends to discourage an immunological interpretation in the majority of cases, it is still true that reactive principles of one sort or another are responsible, even though proteins may not be solely or even directly responsible for the reactions. It would be only reasonable to expect that precipitation reactions between species are due quite as much to the presence of other products of metabolism of species as to their proteins. Although it has been determined that the precipitation in *Iris* is not a calcium oxalate reaction, its exact nature is still to be discovered. In any case, the substitution of another factor or factors for the protein factor does not affect the present evidence, namely, that there is a correlation between the precipitation reactions and success or failure in crossing.

The failure of crosses, in some instances at least, is attributable to the inability of the hybrid embryos to develop in a physiologically hostile environment.

The distinctiveness of *I. pseudacorus* is further confirmed by AUGEM (**1, 2**) in a comparative study of the storage products of the rhizomes of *I. germanica* L., *I. pseudacorus*, and *I. foetidissima* L. He found that the rhizome of *I. germanica* contains saccharose and starch; that of *I. pseudacorus* stores a little saccharose and the levulosan, irisine, but never contains starch; while in the rhizome of *I. foetidissima* are found saccharose, starch, and two levulosans. He concludes that since the three types are so different from one another, physiologically speaking, it may well be because of this fact that crosses between them have so far never produced viable seed. Although in his studies on starch grains REICHERT (**18, 19**) included no mention of any member of the Apogon section, he found a marked difference between the type of grain represented by Pogoniris and Oncocyclus on the one hand and the three bulbous sections on the other (**19**, p. 708).

Summary

1. The precipitation reactions between thirty species, varieties, or hybrids are recorded. They represent, in part, six of the twelve sections of the genus *Iris*.

2. As correlated with breeding evidence, positive precipitation reactions between species usually indicate either a low degree of fertility between such species, or complete interspecific sterility.

3. It appears likely that the presence of a trace indicates too divergent a relationship for a positive reaction of any magnitude. As a rule a trace may be correlated with complete interspecific sterility.

4. A negative reaction between species may indicate close relationship, as shown by successful crosses, or it may indicate extreme divergence between species and be correlated with interspecific sterility.

5. Although decidedly limited, the evidence from gross analyses of storage products and that from microscopic examination of starch grains is in line with the precipitation reactions and the breed-

ing results. As a whole, such evidence supports the present taxonomic grouping, except in the following:

a) *I. prismatica* is divergent from the subsection Sibericae of the Apogon section, as evidenced by gross morphology, breeding results, and precipitation reactions.

b) *I. pseudacorus*, considered in the light of all the available evidence, seems even more distinct from other irises in the Apogon section than its present taxonomic position would indicate.

c) *I. vinicolor* in its precipitation reactions is so similar to the Hexagonae that its inclusion in the group, or with certain members usually assigned to the group, seems warranted.

d) *I. graminea*, in the light of all available evidence, should occupy a rather solitary position.

e) The subsection Laevigatae shares the distinctiveness of its individual member, *I. pseudacorus*. There are positive reactions of varying intensities between the Laevigatae and all other sections and subsections studied, except Vernae. With the Sibericae, however, there is but one positive reaction as contrasted with ten negative reactions, a situation which shows a marked correlation with breeding results, and suggests a comparatively close relationship between the two groups.

6. The precipitation reaction is discussed in the light of chromosome numbers, breeding evidence, and immunological and other phenomena as involved in hybridization and during the ontogeny of hybrid embryos.

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GERMINATION BEHAVIOR OF MAGNOLIA GRANDIFLORA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 442

CLYTEE R. EVANS

Introduction

Magnolia grandiflora (L.) belongs to a very old genus now limited to southeastern North America, southern Mexico, and southern and eastern Asia. It is found in the coastal plain of the southeastern part of the United States. Within this area it grows in rich moist soil on the borders of river swamps and ponds, particularly on hammocks in Florida and fertile rolling slopes along the Mississippi River.

In the literature on seed germination there is very little reference to the behavior of the various species of *Magnolia*. MILLAIS (30) mentions the ready germination of *Magnolia macrophylla* if the seeds are sown immediately after being gathered. He refers to the seeds of certain Chinese species as short-lived, being capable of germination for only a very short time after they are shed. He suggests for seeds of *M. grandiflora* the treatment outlined by BAILEY (2). This method calls for storage of the freshly gathered seeds in dry sand until February, then in moist sand for a week or ten days to loosen the outer fleshy coat. After these coats are removed by washing in water, the seeds are sown in a cold frame. TOUMEY (44) found that the seeds of *M. acuminata* give a germination of only about 3 per cent at maturity, although 68 per cent seem sound. He believes the hard endosperm of these seeds requires several months for the absorption of sufficient water for germination. Observations of a botanist in Florida are to the effect that seeds of *M. grandiflora* germinate in moist situations in the shade of the trees in great numbers in the spring following their shedding in the fall. The writer has observed that seeds of this species when shed from the tree germinate very rarely although they are viable. Probably the limits of the natural range may be related to the germination behavior of the seed.

Nurserymen in the region where *Magnolia grandiflora* grows advise macerating the seeds, stratifying them in moist sand at low temperatures until spring, and then planting. They say that if the seeds dry out they will lie over a year, if they come up at all.

The chemical composition of the seed and the changes taking place in the endosperm and embryo during germination have had almost no attention. WEHMER (**47**) mentions a fat and an ethereal oil, magnoliol, as having been determined in the seed. Alkaloids, glucosides, resin, and saponin have been claimed on evidence not altogether convincing. Studies such as those by TOOLE (**43**) on maize seeds and ECKERSON (**18**) on wheat seedlings interested the writer to attempt to study the chemical changes in the seeds of *M. grandiflora* during normal germination under a given set of conditions. It was believed also that these studies might point the way toward a method of overcoming their dormancy.

Many attempts were made to hasten the after-ripening or to overcome dormancy. A wide variety of conditions was used, as will be indicated in the section dealing with experimental work, but without marked success. A summary of the extensive literature which guided the work is not attempted here.

During the studies of early germination behavior, the writer became interested in the oxidation-reduction mechanisms of the embryos and the food reserves. This interest centered in the activity of oxidase and catalase and in the glutathione mechanism. This compound, which HOPKINS (**24, 25**) discovered and considered a dipeptide of cysteine and glutamic acid, has been reinterpreted by him as a tripeptide including glycine, as well as the two amino acid components first discovered. Since 1921 it has been considered by many workers in animal physiology as having an important rôle in respiration, being present in greatest quantities in tissues having a high metabolic rate. It has been found to be widely distributed in plant tissues also. Up to the present time only slight attention has been given it in connection with studies on seed germination. FIRKERT and COMHAIRE (**20**) have demonstrated its presence not only in active meristematic regions but also in reserves. Of particular interest is the fact that they studied the increase in glutathione in seeds of the pea during absorption of water. This formation of glutathione

has been attributed by VIVARIO and LE CLOUX (**46**) to the hydrolytic decomposition of polypeptide complexes. These studies, together with such work as that of KOZLOWSKI (**28**) on the distribution of non-protein cysteine in meristems and other plant tissues, and that of CAMP (**7**) on the shift in metabolic activity in flower primordia, determined quantitatively on the basis of the nitroprusside color test for glutathione, made it seem particularly desirable to study the glutathione content of *Magnolia grandiflora* seeds during germination. Moreover, interest in compounds containing -SH groups has been stimulated by work done at the Lankenau Hospital in Philadelphia on the healing properties of such compounds as thioglucose, cysteine, and thioglycollic acid. Certain of these have been found to stimulate growth, actually causing an increase in cell division in root tips. It seemed that these compounds might have some significance in the problem of dormancy, such as is exhibited by *Magnolia* seeds.

This paper presents the results of a study of the behavior of seeds of *M. grandiflora* during the preparation for germination and the early stages of the process, with particular reference to the overcoming of dormancy, to the chemical changes taking place during after-ripening, and to the presence and development of oxidizing mechanisms, especially glutathione.

Materials and method

The seeds used in these experiments were of four lots. Lot 1 was 1928 seed received in the pulp nine months after being gathered. Lots 2, 3, and 4 were all 1929 seed. Lot 3 was gathered by the writer in Mississippi in October, 1929; lot 2 was received approximately two months after being harvested; lot 4, seven months afterward. Some seeds of each lot were stored dry at room temperature in glass jars; some of lots 2, 1, and 4, moist at 10°C.; and some of lot 1 were stored dry at 10°. Lot 4 was infected with a green mold when received. Preliminary to storage in damp sterile sand at 10°, the seeds were macerated, after soaking in water 24 hours, and disinfected in $CaOCl_2$ solution.

STRUCTURE.—Observations were made on the gross structure of the seed and seedling and on the course of development of the latter. These were followed by microscopic and microchemical studies of

freehand sections of which camera lucida drawings were made in many instances.

GERMINATION STUDIES.—All seeds in dry storage were macerated and soaked for two hours in $CaOCl_2$ solution prepared as suggested by WILSON (**48**). Tests were run using the seed with the lignified coat on, and others with it removed on one side. Embryos free from the endosperm were set to germinate also. The temperatures used were 10°, 20°, 23°, 29°, and an alternation of 10° and 23°C. The many chemicals used were those suggested by the results of other investigations on problems of dormancy or stimulation.

CHEMICAL CHANGES.—Seeds germinated in the jars of moist sand stored at 10° were studied intensively in an attempt to determine particularly the changes taking place between the splitting of the coat and the emergence of the hypocotyl. In many cases these studies were extended to include the dry seeds of the same lot and seedlings older than the two stages just mentioned. The microchemical tests used were largely the ones for reducing sugars, starch, amino acids, reserve proteins, soluble proteins, fats, and a few others given by ECKERSON (**16**) or TUNMANN (**45**). Free acids were determined by titration with NaOH, using phenolphthalein as the indicator. Neutral red was used to detect localization of changes in reaction in the endosperm and embryo.

OXIDIZING ENZYMES AND GLUTATHIONE.—For the determination of the activity of oxidase, peroxidase, and catalase, qualitative tests suggested by ECKERSON (**16**) were used. These were checked further by quantitative determinations of oxidase according to BUNZELL (**6**) and catalase by the APPLEMAN method (**1**). In testing for glutathione, the nitroprusside method of HOPKINS as used by FINK (**19**) was adopted. Sections of the seeds were heated in dilute acetic acid to the boiling point and then placed in shallow dishes in enough saturated ammonium sulphate to cover them well. Immediately a few drops of a 5 per cent solution of sodium nitroprusside $Na_2Fe(CN)_5NO \cdot 2H_2O$ were added and the material allowed to stand for at least five minutes. Then 1 cc. of ammonium hydroxide, full strength, was added. The appearance of pink to permanganate color was considered to indicate the presence of glutathione. The

depth of the color and the length of time it remained indicated the amount present. The glutathione test was run on seeds of *Ricinus* also.

Results

Structure

Diagrams of the structure of the seeds of *Magnolia* have been published by Brandza (3) and Sargent (41). Brandza interprets the fleshy outer coat as the testa; the inner lignified one as the tegmen; and the membranous innermost one as the remnant of the nucellus. Sargent calls the membranous coat the tegmen, and considers the lignified one and the fleshy one as parts of the testa. Brandza's opinion is accepted here. The fleshy outer coat is made up of three regions: a three-layered epidermis, a wide region of at least six rows of large fleshy cells containing much oil, and within this a layer of small cells forming a sort of inner epidermis. This fleshy coat contains 57 per cent oil and a considerable quantity of reducing sugars. It is traversed by the vascular bundle which leads through the raphe to the chalaza, a region where the coats are undiverged. The lignified coat is made up of radial rows of heavy-walled cells. The nucellus is a membranous layer, brown in color and easily plasmolyzed by the many reagents used in the microchemical tests described elsewhere in this paper. The chalaza and the micropyle lie in a line, the hilum a little to one side of the micropyle.

The endosperm is massive, with a deep groove on one side into which fits a projection of the lignified coat. The cells are large thin-walled ones containing protoplasm packed with both protein reserve bodies (with globoid-like inclusions) and numerous small globules of oil, but no starch grains. The oil content tests 51 per cent. This region gives a striking phytosterol test.

The embryo is extremely small, being 1 mm. in length and 0.4 mm. in diameter. In the freshly gathered seed it shows the same degree of differentiation as it does when the coat splits preliminary to germination, although at this stage it has almost doubled in dimensions. It consists of a hypocotyl and two leaflike cotyledons between which lies a mass of undifferentiated cells, the plumule.

In the germinating seed the shift in the rate of growth is about as

follows. The cotyledons invade the endosperm and extend approximately throughout the length of it before the hypocotyl emerges. In this process one cotyledon usually increases in size more rapidly than the other. Cell contents and cell walls disappear as these cotyledons grow. Soon the hypocotyl takes the lead. First the basal portion pushes out through the endosperm and nucellus; then the upper portion grows rapidly and arches upward, pulling the cotyledons from the remnant of the endosperm. Regularly by this time four secondary roots have appeared in the transition region between root and stem. The cotyledons remain on the plant for four months or more, by which time the plant has acquired seven or eight other leaves.

An interesting change in correlation occurred in 40 per cent of the seedlings developing from seeds previously treated with NaCNS. The upper portion of the hypocotyl pulled the cotyledons out of the endosperm before the emergence of the lower end of the hypocotyl.

Germination tests: Positive results

Tables I and II indicate the type of results with 1929 seed. Each shows the general increase in percentage germination given by these seeds when provided the temperature alternation of 10° and 23°C. The various compounds used in the attempt to increase the percentages of germination or decrease the time required had no marked effect. With one exception the seeds germinated in as high percentage when provided distilled water. This result, the 50 per cent germination secured in one culture with N/5000 zinc sulphate, was probably a chance one as it could not be repeated. No germination was secured in the presence of many compounds tried. A list of these is given.

The time required for germination of seeds with the lignified coats removed from one side at the highest temperature tried, 29°, was 12 days as compared with 30 days required at the alternation and at 20°C. The total percentage was greatest at the alternation.

The time required for germination at 20° or 23° is shortened by storage at 10° in moist sand for 6–10 weeks, whereas a continuance of this storage for 18 weeks seemed less effective, as then the seeds required a longer time to germinate at the same temperature. The increase in time required may be due to the lowering of vitality by

the $CaOCl_2$ used in disinfecting the seeds. Some of it remains in the chalazal region.

Seeds with the lignified coats on when set to germinate at 23° required nine weeks; those with this coat off on one side required from three to four weeks at the same temperature.

TABLE I

PERCENTAGE GERMINATION GIVEN BY SEEDS OF LOT 2 (SEEDS RECEIVED IN PULP; PREVIOUS STORAGE CONDITIONS NOT KNOWN, BUT PROBABLY DRY AT AIR TEMPERATURE FOR TWO MONTHS)

STORAGE CONDITIONS AFTER RECEIPT	TEMPERATURE AT WHICH SET TO GERMINATE	MEDIUM	LIGNIFIED COAT ON OR OFF ON ONE SIDE	PERCENTAGE GERMINATION
10°, moist sand for 30 days	Alternation of 10° and 23°	N/100 KNO_3	Off	15
Dry at room temperature for 30 days	Alternation of 10° and 23°	H_2O	"	30
Dry at room temperature for 30 days	Alternation of 10° and 23°	H_2O	"	25
Dry at room temperature for 30 days	Alternation of 10° and 23°	H_2O	"	35
Dry at room temperature for 30 days	23°	N/2500 $MnSO_4$	"	10
Dry at room temperature for 30 days	"	H_2O	"	5
10°, moist sand for 18 days	"	N/5000 $ZnSO_4$	"	10
" " " " " "	"	H_2O	"	15
" " " " 24 "	"	H_2O_2	"	5
Dry for time	"	1% NaCNS	"	15
10°, moist sand for 24 days	"	N/5000 $ZnSO_4$	"	10
" " " " " "	"	" "	"	15
Dry	"	" "	"	5
"	"	H_2O	"	5
10°, moist sand for 38 days	"	N/5000 $ZnSO_4$	"	10
" " " " 43 "	"	H_2O	"	5
" " " " 18 "	20°	H_2O	On	10
" " " " 38 "	"	H_2O	"	25
" " " " 18 "	"	N/5000 $ZnSO_4$	Off	15
" " " " 40 "	"	N/500 $ZnSO_4$	"	10

Seeds stored in moist sand at about 10° started germinating in 16 weeks and continued this for eight more weeks, when the number began decreasing. Those planted in garden soil in the greenhouse at

a temperature of about 25°C. within a week after being gathered began germinating in 14 weeks.

TABLE II

PERCENTAGE GERMINATION GIVEN BY SEEDS OF LOT 3

STORAGE CONDITIONS	TEMPERATURE AT WHICH SET TO GERMINATE	MEDIUM	LIGNIFIED COAT ON OR OFF ON ONE SIDE	PERCENTAGE GERMINATION
Dry at room temperature for 18 days then in moist petri dishes at 10° for 80 days..	Alternation of 10° and 23°	H_2O	Off	30
Dry at room temperature for 18 days then in moist petri dishes at 10° for 80 days..	Alternation of 10° and 23°	H_2O	"	40
Dry at room temperature for 18 days then in moist petri dishes at 10° for 80 days..	Alternation of 10° and 23°	N/5000 $ZnSO_4$	"	20
Dry at room temperature for 18 days then in moist petri dishes at 10° for 80 days..	Alternation of 10° and 23°	NaCNS	"	25
Dry at room temperature for 18 days then in moist petri dishes at 10° for 80 days..	Alternation of 10° and 23°	H_2O	On	35
Dry at room temperature for 18 days then in moist petri dishes at 10° for 80 days..	Alternation of 10° and 23°	H_2O	Off	40
Dry at room temperature for 18 days then in moist petri dishes at 10° for 80 days..	23°	H_2O	"	30
Dry at room temperature 100 days.....	Alternation of 10° and 23°	H_2O	"	35
Dry for 18 days.....	29°	H_2O	"	10
" " " "	"	H_2O	"	20
" " " "	"	N/800 butyric acid	"	15
" " " "	"	N/1000 acetic acid	"	10
" " 32 "	"	N/500 $ZnSO_4$	"	10
" " 39 "	"	H_2O	"	10
" " " "	"	H_2O	"	10
" " 45 "	"	N/5000 $ZnSO_4$	"	50
" " " "	"	N/100 $NaNO_3$	"	5
" " " "	"	1% NaCNS	"	10
" " " "	"	2% ethylene chlorhydrin	"	5

Germination tests: Negative results

Seeds stratified in moist sand were subjected for 48 hours to a temperature below freezing which caused grains of sand to cling lightly together. All seeds whether their coats were already split or still intact were killed.

Lots 1 and 4 did not germinate at all. Both were apparently sound. The behavior of each differed when stratified and again when set to germinate at 23° from that of lots 2 and 3. Lot 1, the 1928 seed, absorbed less water, 31.2 per cent as compared with 39 per cent absorbed by lot 3. Upon the seeds of lot 4, seeds received from a dealer seven months after they were gathered, blister-like elevations formed, raising the nucellus. These burst after a day or two, liberating a fluid giving the Flückiger test for reducing sugars. Disintegration of the mealy endosperm followed quickly.

Seeds of lot 1 were tested on cellucotton moistened with N/10 HCl, N/10 $HC_2H_3O_2$, N/500 $ZnSO_4$, N/100 butyric acid, N/1000 acetic acid, N/3400 HCl, and 10 per cent dioxygen. Seeds of lots 2 and 3, with lignified coats on or off, gave negative results on N/3400 and N/6800 HCl, N/1600 butyric acid, N/2000 acetic acid, N/5000 $MnSO_4$, 5 per cent KCl, 1.5 per cent acid monopotassium phosphate, 5 per cent glucose, Knop's nutrient solution, sterilized extract of the outer coat, and castor bean lipase plus N/1000 acetic acid. These same seeds failed to respond to soaking for 30 minutes in these reagents: 1 per cent thiourea, 2 per cent KCNS, 2 per cent chloral hydrate, and chloroform in the dilution of one drop in 100 cc. of H_2O.

Embryos removed from the endosperm of dry seeds did not grow on glucose, Knop's solution, sterilized extract of the outer coat, 2 per cent dioxygen, or distilled water.

Chemical changes

Reducing sugars.—In the dry seed there is a trace of glucose in the endosperm directly surrounding the embryo, in the distal half of each cotyledon, and throughout the epidermis of the cotyledons. As the coats split, the first noticeable increase is in the endosperm around the whole embryo. As the seed reaches the stage when the cotyledons are actively invading the endosperm, reducing sugars become more abundant in the periphery of the embryo and occur also

in the plumule and in the cells near the vascular tissues of the hypocotyl. At this stage there is a trace of glucose throughout the endosperm and enough present in some cells near the embryo to give ready formation of osazones. At the time of withdrawal of the cotyledons from the remnant of endosperm, reducing sugars are abundant in the phloem and endodermis of the root and stem, and are present throughout the seedling. In this remnant of the endosperm there is a considerable quantity of reducing sugar, together with much oil and a little protein. Reducing sugars are therefore present in both endosperm and embryo of the dry seed. As the seed prepares for germination these substances increase in both regions, notably in the endosperm near the embryo, in the epidermis of the embryo, in the cotyledons, and in meristem in general. No mashing or packing of cell walls of depleted cells takes place; the walls are digested.

SUCROSE.—Sucrose was found in a few instances with the glucose, but in very small quantities. In all tests greater quantities were found when the hydrolyzing agent was citric acid than when it was invertase. This may indicate the presence of glucosides which were hydrolyzed by the acid but were not affected by the invertase.

STARCH.—Starch was not found in either the endosperm or in the embryo of a dry seed. It was not present in one stratified in damp sand at a temperature of about 10° for 14 weeks or in one with coats barely split. It appears in the mesophyll of the cotyledons as they begin their growth, however, and increases rapidly in amount. Soon the cotyledons become packed throughout, with the exception of an area at the tip and the epidermal cells where none ever appeared. At this stage in some seeds both the endosperm cells lying between the cotyledons of the embryo and others touching it contained starch grains. Before the hypocotyl has pushed out, it also shows considerable starch except in cells of the growing point. It occurs abundantly and in large aggregates of grains in the pith, in pith rays, and in the region of the pericycle and endodermis. Scattered small grains of starch were present everywhere throughout the cortex. By the time the hypocotyl has extended the length of the seed, starch has increased throughout the embryo and become noticeable in some epidermal cells. Root hairs exhibit groups but all growing points are free of them. Starch remains abundant in the embryo until the up-

per part of the hypocotyl begins the rapid growth and arching which draws the cotyledons out from the small remnant of endosperm; then it begins decreasing. At the time of withdrawal of the cotyledons it is present in small quantities in the pericycle, endodermis, mesophyll of cotyledons along veins, and all tissues just below the plumule.

OIL.—There is a great amount of oil in the seed of *Magnolia grandiflora*, 51 per cent on the basis of vacuum-oven dry weight of the seed with coat removed. It is present in both endosperm and embryo in all stages studied, even in those cells of the endosperm touching the embryo, which in the dry seed were found almost free of protein reserves, presumably depleted by the embryo in its development. In the dry seed the oil tends to collect in large globules when mashed out of the cells. In the imbibed seed with coats barely split the oil exhibits the same characteristics and distribution as in the dry one. With the decided growth of the cotyledons, however, followed soon by the pushing out of the hypocotyl, changes in amount and size of the globules become apparent. There is a decrease of oil in the embryo, while in the endosperm it disappears slowly from the cells touching the embryo and a few rows beyond these. At this time there exists a gradation from cells next to the embryo in which there is only a small globule or none at all, through those with large globules, outward to cells with the whole protoplast crowded with them. In these cells which are almost free of oil the protein is completely gone while the cell wall is yet intact. It also finally disappears. Oil globules mashed out of such cells in which oil seems disappearing aggregate in much smaller globules than those from the endosperm of dry seeds, but are not of the size PACK (**37**) describes and considers a translocation form. RHINE (**39**), however, denies a translocation form of fat in the fatty seeds investigated. Oil is present in all cells but is not so abundant as in younger embryos, particularly in those of dry seeds. The decrease in oil seems correlated with the increase in starch and reducing sugars already noted.

RESERVE PROTEINS.—The endosperm cells in the dry grain, with the exception of an area about six cells wide around the embryo, are packed with protein grains, each containing a body which is colorless when treated with picronigrosin. In these cells the protein reserves occur in a decreased amount or are even almost absent in those cells

touching the embryo. Table III summarizes the tests applied to these grains of protein and the amino acid content of the proteins as indicated by them.

The bromine test for free tryptophane gave negative results.

The fewer granules in cells giving the test for soluble proteins are larger than in cells packed with them, and are also much less dense in appearance. In the endosperm of a seed with the cotyledons actively invading it, there is an increasing gradation outward in number of reserve protein grains parallel to that described for oil globules. This same area shows an inverse gradation with reference to soluble proteins.

TABLE III

TEST	AMINO ACID RESPONSIBLE FOR POSITIVE TEST
Xanthroproteic	Tryptophane, Tyrosin, Phenylalanine
Millon's	Tyrosin
Bromine	Histidine
Formaldehyde and H_2SO_4	Tryptophane

SOLUBLE PROTEINS.—By means of the Biuret test soluble proteins were demonstrated. They occurred in small quantities in all seeds tested, whether dry, imbibed, or germinating, and were found most conspicuous in amount in the cells near the embryo in which reserve proteins were scarce. As the seed coat splits, the embryo first shows the test throughout with a deeper color in the root tip, plumule, and epidermis of the whole embryo, and later in the phloem of the hypocotyl and throughout the cotyledons. At the time of emergence of the hypocotyl a striking test is exhibited by the whole of the remainder of the endosperm. The fact that the cotyledons give only a slight test is puzzling. As asparagin gives the Biuret test, these results can probably be interpreted from that standpoint also.

AMINO ACIDS AND ASPARAGIN.—No amino acids could be crystallized out of the dry seed. In tests on the germinating seed, leucine and asparagin were determined, together with an unidentified amino acid which formed broad needle-like crystals. Apparently there was an increase in these with increase in growth of the embryo. No attempt at localization of these acids was made. Quantitative studies could not be carried out because of scarcity of germinating seeds.

REACTION.—The endosperm of a seed with its coats split gave an alkaline reaction with neutral red; the embryo, a very slight acid reaction in the periphery. With further growth this acidity increases in the epidermis of the embryo and becomes noticeable in the end of the hypocotyl and in its vascular regions, as well as in a region of the endosperm surrounding the embryo. The plumule and the pith cells in the upper region of the hypocotyl are still alkaline in an embryo with its hypocotyl 1 cm. in length.

Tests for free acid in seeds ground with sand and transferred to boiled distilled water were made by titration with N/NaOH, using phenolphthalein as indicator. Table IV presents the data. Three seeds were used in each determination.

TABLE IV

CONDITION OF SEED	CC. OF N/NaOH NECESSARY TO NEUTRALIZE FREE ACIDS PRESENT
Coats barely split	0.096
Coats widely split	0.289
Hypocotyls 1 cm. long	0.395

OTHER TESTS: NITRATES.—No evidence of nitrates could be secured in the tests on various stages studied. Seedlings with hypocotyls more than 1 cm. long were not available at the time these were run.

IRON.—Iron was demonstrated in both endosperm and embryo of dry seeds, and showed no change in distribution in one with coats split. In a seedling having the hypocotyl 8 cm. long a striking localization appeared in the root tips and in the cambium of the hypocotyl. Iron was present in the cotyledons but not in such quantities along the veins as could be expected from MOLISCH'S results (**32**).

SILICON.—Tests with phenol crystals failed to show any silicon. This test was carried out in attempting to determine the composition of the heavy walls of the cortical parenchyma and pith of the seedlings.

PHYTOSTEROL.—The protoplasmic contents of the endosperm cells of all seeds gave a decided test for phytosterol with ether and H_2SO_4.

ENZYMES AND GLUTATHIONE

Peroxidase and catalase occurred in small amounts in dry seeds and increased during germination. Oxidase, on the other hand, al-

though not indicated by the microchemical tests on either dry seeds or on those with the coats barely split, was most evident in later stages, appearing in the epidermis of the cotyledons and in the endosperm contiguous to them. Glutathione, however, is present throughout the endosperm of a seed with the coat split. Table V shows the distribution and quantity of glutathione in seeds and seedlings of

TABLE V

DISTRIBUTION OF GLUTATHIONE IN SEEDS AND SEEDLINGS OF MAGNOLIA GRANDIFLORA

MATERIAL	PERCENTAGE WATER CONTENT	COLOR GIVEN BY			
		HYPOCOTYL	COTYLEDONS	PLUMULE	ENDOSPERM
Dry seed	6	−	−	−	−
Soaked seed	37	−	−	−	+
Seed with coats barely split	49	+	−	−	+
Seed with coats well split but hypocotyl not protruding	61	+	+	−	++
Hypocotyls 1 cm. long	Not determined	++	−	−	+++
Hypocotyls 2 cm. long	Not determined	++	Veins ++	+	++++
Hypocotyls 5 cm. long	Not determined	+	+	+	++

Magnolia grandiflora, as indicated by the depth of color of the nitroprusside test.

Glutathione appears first in the lower end of the hypocotyl, then in the phloem of the vascular region. Its first appearance in the cotyledons is in the veins. When it is somewhat abundant in the hypocotyl in general it is present in greatest amounts just above the root cap and in the phloem regions. Parallel tests run on *Ricinus* seeds showed the same general distribution. The amount indicated in the meristem of the root and in the phloem, however, was far greater than in the reserves.

Table VI gives results of the quantitative determination of oxidase activity. Other tests should be run on the same types of materi-

al and on dry seeds without pyrocatechin in the apparatus to measure the autoxidative activity of substances present in them.

Table VII shows the catalase findings reduced to standard conditions. A range is given in each case showing the lowest amount

TABLE VI

OXIDASE DETERMINATIONS

MATERIAL	CC. OF O_2 REMOVED IN 10 HOURS MEASURED IN CC. HG
Six seeds with coats barely split	1.6
Six seeds with cotyledons invading endosperm	7.4
Reagents alone	0.2

TABLE VII

CATALASE ACTIVITY

MATERIAL	CC. OF O_2 LIBERATED FROM 10 CC. OF DIOXYGEN BY 3 SEEDS IN 10 MINUTES	
	LEAST AMOUNT DETERMINED	GREATEST AMOUNT DETERMINED
Dry grains soaked 24 hours	11.80	16.70
Dry grains which would not swell appreciably	3.90	9.40
Stored at 12° for 5 months but not germinating	7.79	10.85
Endosperm showing, seed split	16.70	24.67
Endosperm showing considerably	19.20	27.10
Hypocotyls barely emerging	48.36	54.20
Hypocotyls less than 1 cm. long	66.12	.....
Hypocotyls longer, 2–6 cm	78.76	.....
Endosperms of dry soaked grains	11.35	14.80
Embryos of dry soaked grains	2.96	3.15
Endosperms of seed with coat barely split	13.80	18.75
Embryos of seed with coat barely split	2.96	5.92

found and the highest. No attempts were made to determine the localization of the catalase other than to discover whether it is present in both endosperm and embryo.

Peroxidase seemed localized in the embryo end of the seed in both the dry ones and those with the coats barely split. It, also, increases during early germination stages.

Discussion

Germination tests

The germination tests revealed very little of interest. The fact that seeds and young seedlings cannot endure a light freeze for 48 hours accounts for the narrow range of the tree in nature.

The cause of the delay in germination lies partly, but not wholly, in the lignified seed coat. Removal of this coat allows a low percentage of germination in three or four weeks' time, in seeds which require from two to three times as long with the coat on, or which do not germinate at all. Failure to germinate in such cases is due usually to death of the seed caused by invasion by fungi. Other causes for delay have not been determined. Whatever they may be, they are removed in many seeds by subjecting them to alternating temperatures. Harrington (**23**) discusses the various theories put forward by others, and advances a theory of his own to explain the mechanism of breaking dormancy by alternating temperatures. He thinks that possibly at low temperature there may be an accumulation and actual metabolism of oxygen in a form which becomes available for inception of growth at higher temperatures. Morinaga (**34, 33**) believes we have yet to learn the mechanism or mechanisms of the action of alternating temperatures and their substituting factors. His evidence seems to indicate that the effect is on the embryo. The effect of alternating temperatures on the germination of *Magnolia grandiflora* may be on the nucellus, on the endosperm, on the embryo, or on more than one of these structures. In this connection the 50 per cent reduction of gaseous exchange in *Ambrosia* seeds by the nucellus at 30°C. found by Davis (**11**) is of interest. He believes dormancy in seeds may be caused by the coats restricting the oxygen supply just below the amount requisite for germination at the temperature used. Moreover, he (**12**) induced dormancy in seeds of *Xanthium* by this method. In oily seeds probably oxygen is important in the formation from the fats of more or less unstable compounds which are usable by the embryo in germination.

None of the compounds reported by others as forcing agents had any decided value. It is not known whether or not they penetrated the nucellus. Interesting among these are the following: nitrates,

sulphates, and acetic acid which ROSE (**40**) found to force *Sambucus* seed; dextrose and extract of the fleshy outer coat which IVES (**27**) found to force holly seeds; weak acids which ECKERSON (**17**) found forced *Crataegus* seeds; KNO_3 and $MnSO_4$ for which POPOFF (**38**) and others claim much; ethylene chlorhydrin, thiourea, and NaCNS found of value by DENNY (**13, 14**) in breaking the dormancy of potatoes (possibly through inducing the production of oxidizing enzymes in greater quantity or of greater activity); increased oxygen supply secured through supplying H_2O_2 to the seed bed, as found valuable by SHULL (**42**); and light as found stimulating by GARDNER (**21**).

Why the seeds failed to germinate at all in the presence of some of the reagents is puzzling. In some cases their death was caused by rapid and vigorous growth of mold on the seed in presence of the reagents.

These studies on germination serve to point out the necessity of determining such matters as the permeability of the nucellus and of the endosperm cells to oxygen, CO_2, and various chemicals; the factors influencing the water-absorbing power of the endosperm and the embryo; the effect of other temperature alternations on the germination of freshly gathered seeds and on those variously stored, etc.

CHEMICAL CHANGES AND OXIDATION SYSTEMS

The chemical changes studied in seeds of *Magnolia grandiflora* are especially those occurring between the time of splitting of the seed coat and protrusion of the hypocotyl. The interval between setting to germinate and splitting of the coat was 16 weeks at 10°, and six weeks with alternation of temperature; that between splitting the coat and protrusion of the hypocotyl ranges from six days at 23° to 21 days at 10°C. During this latter interval the trend of both transformations and translocations taking place during the preparation for germination is the same as workers in this field have demonstrated for other seeds. The time required may differ greatly. ECKERSON (**17**) expressed this idea clearly in stating that *Crataegus* goes through the same changes during the 90 days of after-ripening as are telescoped into the first eight days of germination of *Ricinus* as described by DELEANO. IVES (**27**), also, says that the changes

during the long time needed for growth of the immature embryo of *Ilex opaca* are identical with those enumerated by PACK (**35**) as accompanying after-ripening in *Juniperus*. DAVIS (**10**) reported results similar to PACK'S (**35, 36**). She thinks of after-ripening changes as the accumulation of readily usable materials such as sucrose, starch, amino acids, and soluble proteins, whose utilization by the seedling begins with germination and is accompanied by further breakdown of other less easily hydrolyzable reserves. In *M. grandiflora* the accumulation of the starch and soluble proteins in the embryo apparently follows the hydrolysis of reserve proteins and oil in the endosperm cells adjoining the embryo. Although the embryo itself possesses very meager reserves of its own upon which to draw, it seems slow in hydrolyzing its oil reserves. Soluble proteins and reducing sugars in slight quantities are present in it before there is evidence of active changes in the endosperm. It is to be noted, too, that the disappearance of reserve proteins and oil invariably begins next to the embryo, indicating possibly that the embryo somehow initiates these transformations. Questions to be answered here are, how does the embryo effect this, and what stimulates it to activity? These will be discussed later.

Of particular significance and interest are the observations on the disappearance of both protein and oil reserves. The protein bodies swell, become less dense, and disappear entirely. DAVIS (**10**) and LAKON (**29**) report similar changes in size of protein granules in storage cells of other seeds. In the cells depleted of protein granules, soluble proteins are strikingly indicated by the pinkish purple color obtained in the cells by the application of the test. Physiologically the seed of *Magnolia grandiflora* resembles that of *Fraxinus excelsior*, as described by LAKON (**29**), in that in each one a well differentiated embryo grows within the endosperm simultaneously with depletion of proteins stored there. LAKON calls this growth, this preparation for germination, "Vorkeimung," and states that it will occur in six months in moist sand. At the end of this time the seed germinates; that is, the hypocotyl emerges through the inclosing structures. Removing the coats did not overcome the dormancy. Starch appeared in the embryo within ten days after it was put in the sand, but was never present in the endosperm. The reserve proteins became muci-

laginous before disappearing. LAKON thinks they are glyco-proteins which give rise to carbohydrates in their breakdown, as well as to the commonly accepted protein decomposition products.

Clearly in *M. grandiflora* the soluble proteins are derived from the protein reserves. These shorter-chain protein compounds, on being hydrolyzed further to amino acids, pass into the embryo where resynthesis quickly occurs. BROWN and MORRIS (4) consider that the soluble proteins of barley seeds pass very early into the embryo as it swells, and serve there for the development of enzymes. This may be the situation in the seed of *M. grandiflora*, for certainly enzymatic activity of the epidermis of the embryo seems in some way awakened or greatly increased. Of course these proteolytic products of the reserve proteins serve as material for building protoplasm.

Fats also disappear from the endosperm cells adjoining the embryo. Great stores of starch appear in the mesophyll of the cotyledons as this happens. Cell walls also are being broken down. It seems strange that greater quantities of reducing sugar could not be demonstrated in the endosperm and in the cotyledons at this time. The fact that starch can be found in the endosperm cells now and then during disappearance of fat is interesting in this connection. *M. grandiflora* must have very active diastases. If the carbohydrate-like fragments of the fats, whatever they may be, are not used quickly as respiratory or building materials, they seem almost immediately to be condensed and stored as starch. Starch appears later in other parts of the embryo and is always present near growing points but not in them. PACK (36) thinks that in *Juniperus* both fats and proteins contribute to the carbohydrates found.

The acidity accompanying hydrolysis of fats and proteins in *M. grandiflora* can hardly be related in a causal way to the digestion of both, for, in general, lipase has its optimum pH range from 4.0 to 8.6; and plant trypsins from 7.0 to 9.5. Yet it must be remembered that pepsins have an acid optimum range, and that it is short-chain proteins that are abundant and not amino acids. The acidity is probably due to the fatty acids released. Increases in acidity during the germination of oily seeds have been reported time and again, notably by GREEN (22) who discovered lipase, by MILLER (31) in

his work on sunflower seeds, and by IVANOW (26) and ECKERSON (17).

No accumulation of reducing sugar directly precedes growth in an organ during the development of seedlings of *M. grandiflora*. TOOLE (43) reports a correlation between the appearance of reducing sugar in a tissue or organ and its decided enlargement. ECKERSON (18), CHOATE (8), PACK (35), and DAVIS (10) report much reducing sugar present in the hypocotyl or coleorhiza just before the pushing out of the hypocotyl. In *M. grandiflora* reducing sugars can be demonstrated in growing regions but never in large amounts. There seems to be no correlation in time of appearance with the swelling or growth of the organ.

The presence and development of the oxidizing systems is worthy of some comment. The presence of catalase in dry seeds and its increase during the preparation for germination and during the process itself are in agreement with the findings of many investigators. There are, however, notable exceptions. The significance to be attached to the catalase activity of the seeds depends entirely on the function assigned to it. If it is even a rough measure of metabolic activity the increase is significant. Catalase is present in both endosperm and embryo of the seed of *M. grandiflora*.

Although the oxygenase component of oxidase was not indicated by microchemical tests on dry seeds or on those with coats split, it did develop before the emergence of the hypocotyl. ECKERSON (17) reports late appearance of oxidase in seeds of *Crataegus*. TOOLE (43) did not find oxidase in seeds or seedlings of maize. ROSE (40) reports an increase in *Tilia* seeds on germination. Peroxidases, on the other hand, are usually considered to be present in seeds.

As oxygenase was found to be lacking in seeds of *Magnolia grandiflora* which had split their seed coats in preparation for germination, and which must have been respiring, tests for glutathione were made. It was absent in dry seeds but present in all later stages of germination, increasing with development, particularly in growing points and in the phloem until the hypocotyl reached the length of 1 cm. Then it was found decreasing. Tests on *Ricinus* seeds gave similar results. The appearance of glutathione in both these seeds on soaking is in line with the observations of VIVARIO and LE CLOUX

given earlier in this paper. Its distribution in both meristem and reserves agrees with KOZLOWSKI's observations (**28**).

The presence of glutathione in a seed when oxygenase is absent is interesting in view of the fact that it has been considered the most important autoxidizable component of animal cells by eminent physiologists. Possibly other seeds reported as lacking oxidase may possess some such oxidation mechanism. It is significant, too, that iron is found throughout the seed, for late investigations on glutathione accept the idea that iron is a necessary part of the glutathione mechanism.

The fact that glutathione was demonstrated in seeds with split coats hints at the mechanism of absorption of O_2 in the Bunzell oxidase determination on seeds in this stage of development when microchemical tests indicated no oxygenase present. It reconciles the Bunzell determination and microchemical ones.

The sequence of changes during the preparation for germination raises many fundamental questions regarding coordinations of the processes occurring, their concatenations and articulations. One such point is the method of depletion of the endosperm. Does the embryo secrete extracellular enzymes, lipases, ereptases, diastases, to which the living endosperm cells are permeable; or are these enzymes preceded by proteolytic ones produced by the embryo which by killing the cells opens them readily to attack by others? Is it possible that the effect of the stimulated embryo is enzymatic in another sense, in that it removes the products of autohydrolyses already going on in the endosperm cells adjoining it and thus allows their continuance? Many endosperms are capable of self-depletion. Oily endosperms are supposed to be particularly so as they are considered to remain alive throughout artificial or natural evacuation. BRUSCHI (**5**) claims this for the endosperm of *Ricinus* and considers active respiration a necessary condition.

How could the absorption of water accelerate or start these changes in the endosperm? Glutathione appears with the entry of water in the endosperm of *Magnolia grandiflora*. VIVARIO and LE CLOUX consider that in peas it arises from the hydrolysis of polypeptide complexes. The water imbibed may serve to affect the cells in some way so as to bring about the production of glutathione.

Possibly it only modifies the permeability of the cell so that the necessary proteolytic enzyme already present can reach its substrate in the cell. With the production of glutathione, increased respiration is possible if oxygen is available. Do changes in the embryo condition the absorption of water by it, or does the water result as a by-product of increased respiration made possible by the development of the glutathione mechanism?

How do the after-ripening processes in *Magnolia grandiflora* differ from those described for many other seeds? The preparation for germination includes the absorption from the endosperm of a great amount of carbohydrates and products of protein hydrolysis, a process usually reported as taking place in many embryos after the hypocotyl protrudes. This is not strange, for the embryo of this species has very slight reserves and seems slow to attack its oil. The after-ripening includes also a fivefold increase in size of cotyledons, the principal absorbing organs of this embryo.

Summary

1. This paper deals with the behavior of seeds of *Magnolia grandiflora* during preparation for germination and the early stages of the process, with particular reference to the overcoming of delayed germination, to the chemical changes taking place during after-ripening, and to the presence and development of oxidizing mechanisms.

2. Dry storage in the pulp at room temperature seemed almost as advantageous as moist storage of the disinfected seeds at 10° C.

3. Seeds with the fleshy outer coat retained when set to germinate were destroyed by fungi. It was necessary to remove this coat and soak the seeds in $CaOCl_2$ solution in order to control this situation.

4. The lignified coat delays germination but does not prevent it in 5–20 per cent of the seeds. Alternation of temperature was the only means found of securing consistent germination percentages above the one just mentioned.

5. During the preparation for germination the seeds acquire an increased resistance to fungal attack.

6. The endosperm of a dry seed is composed of thin-walled cells containing protoplasm packed with small oil globules and grains of

protein reserves but no starch. Traces of reducing sugars and soluble proteins can be demonstrated in cells near the embryo.

7. The embryo in a dry seed has oil globules in every cell. It shows no protein granules nor starch grains, but a trace of reducing sugar and short-chain proteins.

8. The seed at the time of splitting of the coat has an embryo twice the dimensions of that of the dry seed, but still very small, being on the average only 2 mm. long and 1 mm. wide, lying in an endosperm 10 mm. long and 4 mm. wide.

9. Before the hypocotyl protrudes the cotyledons grow until they are almost as long as the endosperm within which they lie. Their width increases in the same proportion.

10. The chemical changes taking place between the splitting of the coat and the protrusion of the hypocotyl are in general trend the same as have been reported by others as occurring during after-ripening and germination. After-ripening in the embryo includes absorption of food from the endosperm, a process usually reported as occurring during germination but necessitated earlier in *Magnolia grandiflora* by the slight amount of reserve food in the embryo. During the interval just mentioned the water content increases from 49 to 61 per cent.

11. Oils decrease in amount in the endosperm, the process beginning near the embryo.

12. Soluble proteins increase in endosperm cells near the embryo and in the embryo itself when reserve proteins disappear in these endosperm cells.

13. Starch appears in the cotyledons in striking quantity when the reserves of the endosperm begin disappearing. No detectable amount of oil in the embryo disappears previous to the appearance of the starch.

14. Traces of reducing sugars as well as of soluble proteins occur in the dry seed. Increases in these in the embryo as germination begins cannot be correlated in time with the beginning of rapid growth of these organs.

15. Iron is present in all parts of the seed and abundant in root tips of the seedling.

16. The epidermal cells of the embryo increase in acidity as indicated by depth of color with neutral red; and the contents of endosperm near these cells change from alkaline to acid.

17. Oxidase is not demonstrated by microchemical tests in seeds with the coat barely split, but becomes evident before protrusion of the hypocotyl and increases during later development.

18. Glutathione appears in the seed during soaking in H_2O. It is demonstrable first in the endosperm, then throughout the embryo as germination progresses. It is present previous to development of the oxidase mechanism.

19. Peroxidase and catalase are present in the dry seed, increasing with germination.

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EFFECTS OF SULPHURIC-ACID DELINTING ON COTTON SEEDS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 443

AUVAL HESTER BROWN

(WITH FOUR FIGURES)

Introduction

Cotton seeds vary greatly in their rate of germination (11). This fact has been observed by cotton growers for a number of years; but the reasons for their failure to germinate have been the subject of much comment and diversity of opinion (2, 5, 11) among agricultural research workers. Poor germination frequently necessitates the replanting of whole fields several times (11), or else reduces the yield per acre. The consequent tardiness of vegetative growth also makes fruiting difficult (13) in boll-weevil infested areas, and as a result, the yield is decreased materially.

The rôle of the seed coat in delayed germination among certain plants has been recognized (6, 7) for some time. However, few important laboratory studies have been made in connection with the germination of cotton seeds. On the other hand, the experiment stations of the various cotton-producing states and countries have been active in their efforts to secure a uniform rate of germination, and to disinfect the seed coat which carries parasites (5) in the form of fungal spores and bacteria. With the latter object in view, DUGGAR (8) used the sulphuric-acid treatment as a control measure in his efforts to check anthracnose in Alabama as early as 1911. Complete sterilization of the seed coat was reported and the disease was checked so far as external infection was concerned. LEHMAN (10) also used this method of delinting cotton seeds for the same purpose and obtained similar results. Concentrated sulphuric acid was found superior to such solutions as copper sulphate, mercuric chloride, and formaldehyde, since the fuzz on the surface of the seed did not permit the latter solutions to make contact with it, thus a large portion

of the surface was untouched by the reactions of these disinfectants. The difficulty of applying the sulphuric acid to the seed coat made the method impracticable until BROWN (4) began his studies of angular leaf spot on Pima Egyptian cotton. So successful was the treatment as a disinfectant for this seed-borne parasite, that a machine (5) was devised for the cooperative treatment of cotton seeds on a large scale at a very low cost. This improvement over the tedious methods (8) employed by former workers has stimulated its use in regions where long staple cotton is grown and where angular leaf spot is much more prevalent than on upland cotton. Recently the use of concentrated sulphuric acid on upland varieties has proved helpful both as a disinfectant and as an aid to germination. Experiment stations, particularly in Arkansas (9) and South Carolina (3), have employed H_2SO_4 with these purposes in view. The results obtained by the South Carolina station show a marked increase in the yield of cotton per acre in field tests.

This investigation was undertaken with the purpose of making a careful laboratory study of the effects of H_2SO_4 on the germination rate of four short staple and two long staple varieties of upland cotton commonly grown in Oklahoma, and to study the effects of the treatment on the yield in the field tests.

Materials and methods

Samples of six varieties of cotton seeds were obtained from the Oklahoma experiment station in November, 1927. Four of these varieties, Half and Half, Mebane, New Boykin, and Oklahoma Triumph 44, were short staple upland cotton; the other samples, Acala and Delfos, were long staple upland varieties. The seeds were stored in the basement of a well ventilated building which was heated moderately by steam. Laboratory experiments were commenced the following April. Each variety was tested separately to determine the minimum, optimum, and maximum time limit for treatment by concentrated sulphuric acid of specific gravity 1.84. Small samples of the seeds were placed in beakers and about three times their volume of concentrated H_2SO_4 was added. The mixture of seeds and sulphuric acid was stirred continually with a glass rod to insure uniform contact of the delinting agent with the lint, and a Centigrade thermom-

eter was inserted to permit observation of temperature changes during the delinting process. The duration of the H_2SO_4 treatment was varied, six time-intervals being used for each of the six kinds of seeds used, the intervals being 5, 10, 15, 20, 25, and 30 minutes respectively. At the expiration of a given period of treatment, the mass of delinted seeds and sulphuric acid was transferred to a Buchner funnel and the H_2SO_4 drained off rapidly. Tap water was run freely over the seeds and through the funnel to remove the acid from the surfaces of the seeds. This operation was performed quickly, and with excess water, to prevent much rise in temperature resulting from the dilution and hydration of the concentrated acid. The delinted seeds were then washed for a few minutes in $Ca(OH)_2$ to neutralize any adsorbed sulphuric acid which had not been removed by the previous washing. Tests were made with litmus paper after three minutes to make sure that the seeds and solution were no longer acid.

To remove the poor quality of shriveled seed from the material, the washed seeds were placed in a pneumatic trough where the faulty light ones were separated from the heavier ones. The latter were placed between filter papers and dried to constant air-dry weight. Samples treated in this manner were designated lots a, b, c, d, e, and f, depending on the duration of the H_2SO_4 treatment. Fifty seeds from each lot were placed between moist filter papers in appropriate germinators. The control for each variety of cotton seed used consisted of fifty undelinted seeds, referred to in this discussion as lot I. Observations were made daily, and records of the germination rate were kept to determine the effectiveness of the treatment in hastening development of the seedlings. Similar experiments on the same varieties of cotton were also conducted in the botanical laboratory of the University of Chicago during the summer of 1929.

Field tests of the germination rate were made in an open field at McAlester, Oklahoma, and in the University of Chicago greenhouse. Delinted seeds were allowed to dry before planting; and in each instance, undelinted ones of the same variety were used as a check. The greenhouse plots were planted in drills, and also in pots which were sunk in soil. The field tests at McAlester, made in 1931, included a study of the yield from plants derived from delinted and undelinted seeds. The plants were cultivated according to standard

field practices, and harvested at the normal time. The growth and yields are expressed in pounds of cotton produced per acre.

The intake of moisture by cotton seeds was measured during the summer of 1929 on one variety, Oklahoma Triumph 44. Lots of ten seeds each were selected, one with lint on, one with lint removed with sulphuric acid, and one with the brown shell removed from the embryo and endosperm. Each lot was weighed at constant air-dry weight and placed between moist filter paper in germinators. Weighings of each lot were made at the end of 1, 3, 5, and 22 hours respectively to determine the amount of moisture taken in by the cotton seeds under each of the three conditions. Observations were stopped at the end of 22 hours, when the seeds with brown shells removed from the embryo and endosperm had germinated. The percentage of moisture taken in was calculated and recorded.

Results

The delinting process reduced the time required for cotton seeds to germinate in both laboratory and field tests. Results obtained in these experiments varied with the six varieties. Data secured from tests made in the laboratory, in the open field, and in greenhouse plots are given in tabular form. The results with the Acala variety are shown in table I.

The yellow color of the linters on the seeds of the Acala cotton, and the great number of light-weight seeds removed by flotation during washing, indicated low quality seed, and many small inferior seeds were actually found among them. The poor condition of the seed related to poor crop yield, injury in the field owing to climatic factors, or improper storage of seeds after ginning (9). The low percentage of seeds germinating in the control at the end of the sixth day is therefore mainly due to the fact of this inferior quality. The delinting process was probably continued too long for long staple cotton, which was more uniformly delinted by ginning than the short staple varieties used in this work. Injury was observed in lots b to f inclusive, which were subjected to treatments of longer duration than 5 minutes, the limit of the delinting process used in lot a.

Temperatures observed during the delinting process were far below the thermal death point of cotton seeds (11) and apparently were of no consequence in the results obtained.

Seeds of the variety Delfos, also a long staple upland cotton, were of better quality than those of Acala (table I). The data reported in table II show not only a higher percentage of germination in the control, but a definite increase in the rate and percentage of germination of the delinted seeds. Five minutes was found to be the optimum time for treatment in this variety. Although a higher

TABLE I

EFFECT OF DELINTING ON PERCENTAGE OF GERMINATION IN FIFTY SEEDS OF ACALA VARIETY OF COTTON; 6/25/29

LOT	DELINTED BY CONCENTRATED H_2SO_4 (MINUTES)	TEMPERATURE DURING DELINTING (°C.)	PERCENTAGE GERMINATION			
			3 days	4 days	5 days	6 days
a	5	37	6	16	18	48
b	10	38	.....	.....	6	18
c	15	36	.....	.....	10	12
d	20	36	10	10	10	10
e	25	34	Attacked by mold			
f	30	34	2	4	8	10
1	Control	.....	.....	6	18	36

TABLE II

EFFECT OF DELINTING ON PERCENTAGE OF GERMINATION IN FIFTY SEEDS OF DELFOS VARIETY OF COTTON; 6/2/29

LOT	DELINTED BY CONCENTRATED H_2SO_4 (MINUTES)	TEMPERATURE DURING DELINTING (°C.)	PERCENTAGE GERMINATION			
			2 days	3 days	4 days	5 days
a	5	35	52	80	88	90
b	10	35	30	66	78	80
c	15	35	32	66	82	82
d	20	38	68	90	92	92
e	25	34	48	80	88	90
f	30	32	68	78	84	84
1	Control	.....	.....	30	68	82

percentage of germination was obtained in lot d, the radicles and seed leaves showed injury by the delinting agent. All lint was removed in 5 minutes, and the surfaces of the seeds were sterilized, which accomplished the purposes of the delinting process. In both of the long staple cottons used, therefore, treatment exceeding 5 minutes is likely to have an injurious effect upon the embryos from penetrating sulphuric acid.

Half and Half variety of cotton produced lint of inferior quality which was easily removed by the delinting process. Optimum duration of treatment by concentrated H_2SO_4 was found to be 5 minutes. Slight injury from the delinting agent was observed in lot d, and this was very pronounced in lots e and f (table III). Pot cultures grown in the greenhouse showed an increase in the rate of germination in lot c (fig. 1, right), which was delinted 15 minutes by concentrated H_2SO_4; and also in lot 1 (fig. 1, left), which was the control. The plants were photographed 7 days after planting. The tendency of

TABLE III

EFFECT OF DELINTING ON PERCENTAGE OF GERMINATION IN FIFTY SEEDS OF HALF AND HALF VARIETY OF COTTON; 6/29/29

LOT	DELINTED BY CONCENTRATED H_2SO_4 (MINUTES)	TEMPERATURE DURING DELINTING (°C.)	PERCENTAGE GERMINATION			
			2 days	3 days	4 days	5 days
a	5	36	74	96	94	100
b	10	36	84	92	92	92
c	15	36	86	94	94	96
d	20	38	76	90	92	92
e	25	34	76	80	82	86
f	30	36	44	64	80	84
1	Control	.....	.....	6	84	98

the undelinted tegmen to inclose the seed leaves after the hypocotyl has raised them above the soil can be seen in figure 1 (left). The sulphuric-acid treatment enables the cotyledons to escape (4) more readily from the investing membranes.

The Mebane sample was in poor condition, probably because of improper methods of harvesting or storing (9). Many faulty shriveled seeds were found when delinted by concentrated H_2SO_4. A great number of these were removed by flotation in the washing process, but the remainder gave inferior germination because they were severely attacked by fungi when placed in germinators. The fungal attacks were by species of *Rhizopus*, which according to LEHMAN (10) were caused by spores and mycelium that were within the seed coat and could not be reached by surface disinfectant. TOOLE and DRUMMOND (11) attributed the growth of these fungi to the moisture content of the seed. In work done with Texas cotton, it

was found that if the moisture content of stored seed fell below 10 per cent, there was a gradual increase in the tendency of the sample to mold and decay during germination. Fungal growth was more

FIG. 1.—Cotton seedlings, variety Half and Half; *A* (left), rate of field germination of 50 undelinted seeds; *B* (right), rate of field germination of 50 delinted seeds. Photographs taken 7 days after planting.

TABLE IV

EFFECT OF DELINTING ON PERCENTAGE OF GERMINATION IN FIFTY SEEDS OF MEBANE VARIETY OF COTTON; 6/27/29

LOT	DELINTED BY CONCENTRATED H_2SO_4 (MINUTES)	TEMPERATURE DURING DELINTING (°C.)	PERCENTAGE GERMINATION			
			3 days	4 days	5 days	6 days
a	5	36	4	8	12	Mold
b	10	37	14	14	14	"
c	15	37	6	8	16	"
d	20	36	6	20	20	"
e	25	35	16	16	18	"
f	30	33	18	18	20	"
1	Control	.....	Attacked by mold			

abundant on the control than on delinted seeds, but was present the sixth day in every lot because conditions for its growth were exaggerated in the germinators.

The optimum time limit for treatment by concentrated H_2SO_4 in the New Boykin variety was 5 minutes. All treatments beyond this

duration showed injury, both to the seed coat, which was pitted and granular and often stained the walls of the germinator a dark brown, and to the radicles which were dwarfed and irregular in shape. The control gave a low percentage of germination caused by the growth of mold probably resulting from spores and mycelium borne in the lint, since the fungus did not occur on the delinted seeds.

TABLE V

EFFECT OF DELINTING ON PERCENTAGE OF GERMINATION IN FIFTY SEEDS OF NEW BOYKIN VARIETY OF COTTON; 6/29/29

LOT	DELINTED BY CONCENTRATED H_2SO_4 (MINUTES)	TEMPERATURE DURING DELINTING (°C.)	PERCENTAGE GERMINATION			
			2 days	3 days	4 days	5 days
a	5	35	64	78	84	90
b	10	35	34	42	48	64
c	15	35	54	72	74	82
d	20	37	12	12	22	34
e	25	35	28	40	54	56
f	30	35	28	38	52	52
1	Control	.....	.....	4	56	76

TABLE VI

EFFECT OF DELINTING ON PERCENTAGE OF GERMINATION IN FIFTY SEEDS OF OKLAHOMA TRIUMPH 44 VARIETY OF COTTON; 6/22/29

LOT	DELINTED BY CONCENTRATED H_2SO_4 (MINUTES)	TEMPERATURE DURING DELINTING (°C.)	PERCENTAGE GERMINATION			
			2 days	3 days	4 days	5 days
a	5	37	82	94	94	94
b	10	37	78	86	96	96
c	15	42	74	86	94	94
d	20	40	96	100	100	100
e	25	41	94	100	100	100
f	30	41	26	52	62	62
1	Control	.....	.....	16	74	94

The optimum duration of treatment by concentrated H_2SO_4 in the Oklahoma Triumph 44 variety was 20 minutes. Figure 2, at the left, shows the appearance of seeds before the process of delinting. Figure 2, at the right, shows the seeds from the same sample which were given treatment for 20 minutes. Figure 3 shows the difference in the rate of germination when both lots had been under germinating con-

ditions for 48 hours. Seeds delinted by sulphuric acid for 10 and 25 minutes respectively also showed that the duration of treatment could be lessened or extended in this variety without influencing the results materially. Lot f, which was given treatment for 30 minutes, showed definite injury to both the seed coats and the radicles of the germinating seeds. In other lots the rate of germination was de-

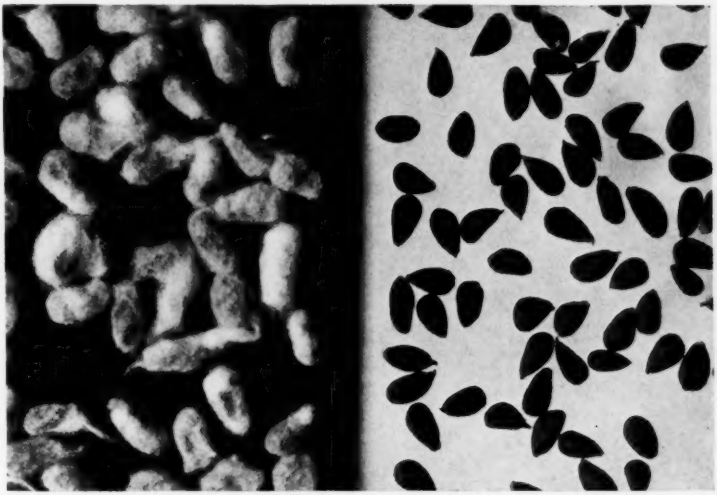

FIG. 2.—Cotton seeds, variety Oklahoma Triumph 44. Left hand side shows appearance of seeds before process of delinting; right hand side shows sample of same seeds after delinting treatment by concentrated sulphuric acid for 20 minutes.

cidedly more rapid in the treated seeds. The germination in delinted seeds was one to three days in advance of the controls.

The test for the effect of delinting upon the yield was conducted on sandy loam soil of average fertility under boll-weevil conditions where early germination is essential. Both plots for delinted and control were planted in rows 150 yards long and 3 feet apart, and the results (table VII) were based on the number of pounds of seed cotton produced per acre. The yields were high for this locality, in which the cotton was grown for the 1931 season. Oklahoma Triumph 44 gave the highest percentage of germination, produced the best fruiting habit, and yielded the highest average of seed cotton

per acre. The rate of germination in the field was based upon the appearance of the cotyledons above the ground. In each plot, delinted seeds germinated two days earlier than the control; and, even until harvest time, both the vegetative growth and the fruiting habit of the seedlings were much better than those from undelinted seeds.

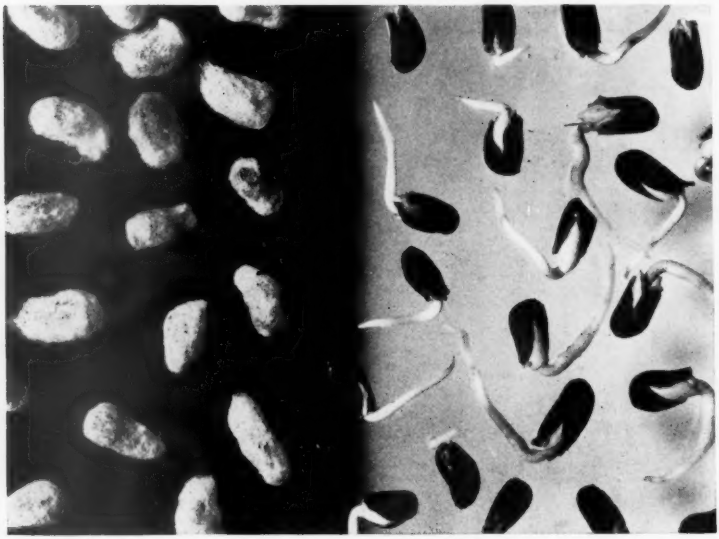

FIG. 3.—Cotton seeds, variety Oklahoma Triumph 44, showing rate of germination of delinted and undelinted seeds. Left hand side of figure shows undelinted seeds under germinating conditions for 48 hours; right hand side shows delinted seeds under germinating conditions for 48 hours.

Further proof of the shorter time required for the germination of delinted seeds is shown by the results of soil tests conducted in the greenhouse. The results shown in table VIII were obtained from a plot of ground in the University of Chicago greenhouse. Counts were made daily from the fourth to the ninth day, counting all seedlings the cotyledons of which had opened, and basing the percentage of germination on the total number of seeds planted. Variations were more noticeable in the varieties Half and Half and Oklahoma Triumph 44. Observations made 15 days after planting showed that

seedlings produced from delinted seeds were more uniform and vigorous than those of the control of the same variety.

Accurate weights, to a thousandth of a gram, showed that the lint was a hindrance to the absorption of water by the seeds. The rapid

TABLE VII

EFFECT OF DELINTING COTTON SEED BY SULPHURIC ACID FOR 5 MINUTES ON YIELD OF SIX VARIETIES (FIELD TESTS); SEEDS PLANTED 5/10/31

VARIETY	RATE OF GERMINATION (DAYS)		FRUITING HABIT		YIELD IN LB. PER ACRE	
	DELINTED	UN-DELINTED	DELINTED	UN-DELINTED	DELINTED	UN-DELINTED
Acala	3	5	Good	Good	967.80	612.94
Delfos	3	5	"	"	1064.58	838.76
Half and Half	3	5	"	"	1032.32	1029.10
Mebane	3	5	"	"	806.50	677.46
New Boykin	3	5	"	"	1032.32	709.72
Oklahoma Triumph 44	3	5	"	"	1096.84	838.76

TABLE VIII

EFFECT ON RATE AND PERCENTAGE GERMINATION OF DELINTING SIX VARIETIES OF COTTON SEEDS BY CONCENTRATED H_2SO_4 TREATMENT FOR 20 MINUTES (GREENHOUSE TESTS)

LOT	VARIETY	DATE (1929)	TREATMENT	PERCENTAGE GERMINATION (DAYS)					
				4	5	6	7	8	9
a	Acala	6/25	Delinted	.....	.....	55	85.0	95.0	95.0
b	Delfos	6/28	"	.....	.....	45	65.0	67.5	85.0
c	Half and Half	6/28	"	80	85.0	95	95.0	95.0	95.0
d	Mebane	6/28	"	.....	.....	25	30.0	57.5	80.0
e	New Boykin	6/28	"	.....	.....	30	55.0	80.0	87.5
f	Oklahoma Triumph 44	6/22	"	50	77.5	90	90.0	90.0	90.0
1	Acala	6/25	Control	.....	.....	.....	10.0	75.0	90.0
2	Delfos	6/28	"	.....	.....	.....	25.0	50.0	62.5
3	Half and Half	6/28	"	.....	.....	45	65.0	67.5	85.0
4	Mebane	6/28	"	.....	.....	.....	20.0	50.0	60.0
5	New Boykin	6/28	"	.....	.....	.....	27.5	72.5	80.0
6	Oklahoma Triumph 44	6/22	"	.....	.....	50	67.5	67.5	72.5

increase in absorption by seeds with the brown shell removed from the embryo and endosperm gave a much higher rate of germination than seeds with either smooth seed coats or lint. At the end of 22

hours, seven out of ten seeds with shells removed had germinated; and five out of ten delinted seeds had started to germinate; whereas the seeds with lint were only slightly swollen.

Discussion

The results of this investigation show that delinting cotton seeds by concentrated sulphuric acid gives both a higher percentage and a more rapid rate of germination. The delinted seeds make closer con-

TABLE IX

ABSORPTION OF WATER BY OKLAHOMA TRIUMPH 44 VARIETY OF COTTON SEEDS

SEED TREATMENT	WEIGHT OF 10 SEEDS (GM.)	AMOUNT OF WATER ABSORBED							
		1 HOUR		3 HOURS		5 HOURS		22 HOURS	
		GM.	PER CENT	GM.	PER CENT	GM.	PER CENT	GM.	PER CENT
1. With lint	1.2100	0.0312	2.5	0.0534	4.4	0.0721	5.9	0.3846	31.7
2. Lint removed with H_2SO_4	0.9657	0.0390	4.04	0.2710	28.6	0.494	51.1	0.756	78.2
3. Shell removed from embryo	0.8261	0.2710	20.4	0.4232	51.2	0.5889	71.3	1.1065	133.9

tact with the water of the soil, thus germinating more readily. The intake of water by seeds on filter paper in germinators was much higher in delinted than in undelinted seeds. Exclusion of water by the seed coat was regarded by CROCKER (6) as the real cause of delay in germination of *Axyris amaranthoides* and *Abutilon avicennae*. He thought that the seed coats of many others, as for instance the hard seeds of the Leguminosae and Malvaceae, prevented the embryos from absorbing sufficient water for germination. Slow water absorption seems to be the cause of delayed germination in the undelinted seeds of cotton. The sharp contrast between the rate of germination of treated and untreated seeds of variety Oklahoma Triumph 44 is illustrated by figure 3. The two samples were placed under germinating conditions at the same time. The undelinted seeds are swollen but not germinated, whereas the delinted seeds show 100 per cent germination. Accurate measurements of the absorptive rate of water by these seeds is shown in figure 4.

Field tests show that the seed coat is a hindrance to germination.

The seedlings produced from delinted seeds in the open field and in the greenhouse plots appeared above the soil unhampered by the tegmen. On the other hand, the linted tegmina cling to the soil and prevent the unfolding of the cotyledons. BROWN (4) found that seed coats covered with lint were adapted to holding fast to the soil, in this way delaying germination. Another way in which seedling development was delayed was by the adherence of the tegmen to the cotyledons for several days after they were above the ground. This is illustrated by figure 1, which shows the linted tegmen around the cotyledons, while the right hand side of this same figure shows the increased growth of seedlings from delinted samples of the same variety, Half and Half. Both pots were photographed 7 days after the date of planting. WATKINS (12) reported that cotton seedlings produced from seeds treated with superphosphate paste had difficulty in splitting the tegmen. The treatment had so hardened the covering that, although rooted, the young plants were unable to unfold the seed leaves and make their appearance, and as a result were killed. Sulphuric-acid treatment of seeds makes the seed coat more porous and increases the absorption of water to such an extent that the coat is easily removed by the seed leaves soon after their appearance above the soil. The delinting process can be accomplished by concentrated sulphuric acid without injury to the embryo, provided

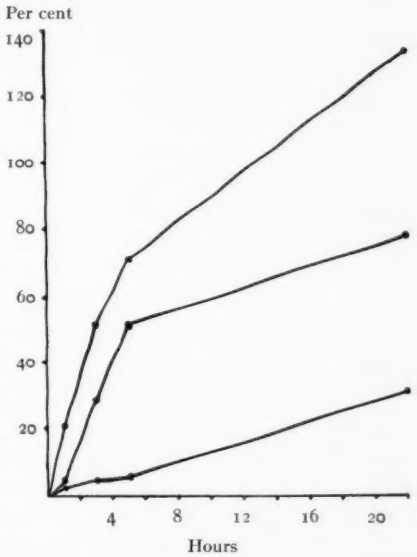

FIG. 4.—Upper graph, absorptive rate of water by cotton seeds with brown shell removed from embryo and endosperm; center graph, same with lint removed with H_2SO_4; lower graph, same with lint on seeds.

the treatment is not too prolonged. The optimum duration of treatments by the delinting agent was found to vary slightly with the six varieties of cotton studied. Five minutes was found to be sufficient time to remove the lint and to sterilize the surface of the seed coat. Light shriveled seeds could be detected and removed by flotation after this period; hence for practical purposes this duration of treatment is adequate.

Crude and tedious methods used by early workers (**8**), and the danger in handling sulphuric acid (**5**) in open vessels seemed to discredit this form of delinting cotton seeds. When DUGGAR (**8**) first used the method in Alabama in 1911, the process was slow and only partially destroyed the anthracnose, which was an internal as well as an external parasite. The practice was abandoned until 1922, when interest was renewed (**9**) to secure more rapid germination and to sort the heavy from the light seeds by flotation. A very practical machine was designed, the initial cost of which can be met by cooperative and extensive use (**5**). It can be made to serve several purposes; for instance, when grains are disinfected with certain dusts, the machine serves both for dusting and delinting (**5**).

The method of delinting cotton seeds by sulphuric-acid treatment is superior to that of cutting the lint by oil-mill machinery. In the first place, the removal of the lint is more uniform and much more complete. Seeds of varying size, delinted by saws operated mechanically, are frequently crushed and cut, because no provision is made for the irregularities of form in that method of delinting. The concentrated sulphuric acid reacts with the cellulose of the lint very quickly, and the result is a smooth seed that is entirely intact and with no injury to the embryo. The linters removed by oil-mill machinery (**1**) may be saved by that process; but their commercial value has depreciated, and they are no longer of importance owing to the inferior quality of the lint so obtained.

The sulphuric-acid treatment produces smooth delinted seeds which can be planted by machine to better advantage than can those with seed coats covered by lint (**9, 5**). Planters of different types must be used so that the seeds will not be crushed by the revolving plates, for delinted seeds will feed through the machine with as much ease as other smooth seed like corn and beans. The delinted seeds may be spaced accurately with check-row devices. Uniformity

in the rate of germination gives vigorous seedlings which permit early cultivation. This promotes better growth, an important factor in boll-weevil infested areas (**13**) where early cotton is desirable to increase the yield. The difficulty of cotton planting when the soil is cold from excessive rains can be partially overcome by this treatment, because delinted seeds planted a few days late germinate rapidly and make up for the delay in planting. In this way conditions for early fruiting may be produced to offset losses from the boll weevil.

Summary

1. Tests of the value of delinting the seeds with H_2SO_4 before planting have been made with six varieties of cotton used widely in Oklahoma.

2. Delinting of the seeds by means of this agent gives a higher percentage and an increased rate of germination. No injury to the embryo results, providing the treatment is not prolonged.

3. The successful optimum duration of the delinting process was 5 minutes for long staple upland varieties, and could be extended to 20 minutes without injury in short staple cotton seeds.

4. Yields from mature plants derived from delinted seeds exceeded those from undelinted by an average of 21.4 per cent, under the same field conditions.

5. Smooth delinted seeds are well adapted to agricultural practices with modern machinery; and by the cooperative use of a delinting machine, the cost is moderate.

Grateful acknowledgments are made to Dr. Charles A. Shull of the University of Chicago for his helpful criticisms, to L. L. Ligon of the Oklahoma Agricultural Experiment Station for seeds of the six varieties used, and to Edward Brown for assistance in care of field plots.

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FLOWER BUD FORMATION IN THE CONCORD GRAPE

JOHN C. SNYDER

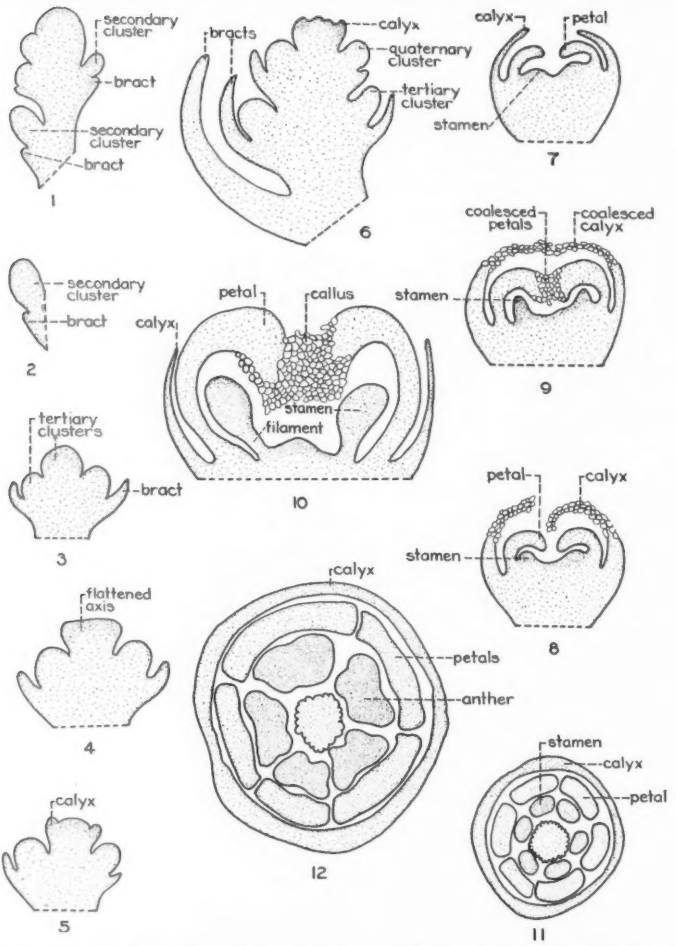
(WITH TWENTY-TWO FIGURES)

Introduction

Most literature concerning the morphology of the flower of the American grape, *Vitis labrusca*, deals with the late stages of development rather than with the origin of the inflorescence. DORSEY (**5, 6**), BEACH (**1, 2**), and BOOTH (**3**) studied the later stages of flower development, and the recent work of PARTRIDGE (**10**) and COLBY (**4**) consists primarily of field experiments. Although GOFF (**8**) states that "embryo flowers" are discernible in the grape bud during the previous season, and JOHNS (**9**), working with the grape in 1925, reports small "masses of flowers" forming about October 30, there is little detailed information concerning the initiation and early development of the inflorescence. The studies which are presented in the following pages attempt to supply this information.

Materials and methods

In the grape, a group of two to five buds is present at each node, the group being referred to as an eye. Although each bud of an eye may produce a shoot, it is more common for only one shoot to develop at a node. One of the several buds, being larger and structurally more advanced than the others, is referred to as the primary bud and the progressively less developed buds are referred to as secondary, tertiary, and quaternary buds respectively (fig. 21). The primary buds from three to five shoots were examined, being collected at weekly intervals during the growing season and at triweekly intervals during the dormant period. Projection drawings were made from longitudinal serial sections of a sufficient number of buds to show their chronological development for the year 1931–1932. The serial arrangement of the buds on the shoots was maintained during each collection, so that comparisons could be made of buds



FIGS. 1–12.—Fig. 1, primordial cluster with secondary clusters subtended by bracts. Fig. 2, secondary cluster and bract removed from cluster shown in fig. 1. Fig. 3, secondary cluster subdivided to form three tertiary clusters. Fig. 4, secondary cluster showing three tertiary clusters (axes of third order), central one showing a flattening, the first evidence of flower formation. Note determinate type of inflorescence of secondary clusters shown in figs. 4–6. Fig. 5, calyx evident in central flower. Fig. 6, secondary cluster similar to that in fig. 3 except that central tertiary cluster has subdivided to form three quaternary clusters. Calyx evident in central quaternary cluster. Fig. 7, flower showing calyx, petals, and stamens. Note that calyx and petals have started to form cap over floral axis. Fig. 8, flower with calyx showing considerable proliferation. Fig. 9, flower with calyx and petals coalesced. Fig. 10, flower with calyx cap ruptured. Petals show marked coalescence and stamens have differentiated into anther and filament. Fig. 11, flower in cross-section showing calyx, petals, and stamens. Fig. 12, flower in cross-section showing calyx and petals, and anthers in quadrilobed condition. ×8.

from one position collected at any one time with buds from other positions and other times of collection. An acetic-formalin-alcohol killing fluid was used. The penetration of the reagents and the sectioning of the buds were greatly facilitated by shaving off one face of each eye and removing the bud scales and hairs. Sectioning was further facilitated by treating the buds with hydrofluoric acid and soaking the paraffin-imbedded specimens in warm water for 1–8 days. Fast-Green dissolved in 95 per cent alcohol proved very satisfactory.

Initiation and development of cluster

Lateral buds arise as protuberances near the tip of a shoot and are inclosed by bud scales. The apical primordium of each bud elongates and in turn produces several lateral outgrowths, the primordia of bud scales, leaves, and inflorescences (fig. 13). By the time there are approximately four such outgrowths on the axis of the bud, two of them, located alternately near the base of the axis, have elongated and become somewhat pointed. These can be identified at this stage as primordial leaves (fig. 13). Additional lateral primordia appear concurrently with elongation of the axis. The distal primordia are produced in pairs, one on either side of the bud axis, differing from those at the base of the axis, which are single. The lobes of each pair, although alike in the initial stage, soon acquire different shapes, one taking on the characteristic shape of a leaf primordium and the other becoming obovate. This obovate primordium is the first evidence of a cluster (fig. 13). While differentiation from leaf to cluster primordium is taking place, axillary buds are being laid down in the axils of the two previously formed primordial leaves (fig. 13).

A diagram (fig. 22) is presented in order to clarify the nomenclature necessary in describing a cluster. The inflorescence of the grape is a racemose panicle. The main axis (*a*) bears lateral axes of the first order (*b*), and these in turn bear lateral axes of the second and third orders (*c*, *d*). The lateral axes arising near the base of the rachis are longer and more profusely branched than those arising from the more distal regions. The degree of branching gradually decreases toward the apex, so that the distal part of the cluster usually consists of simple axes. The cluster of fruit borne by each axis is referred to as a secondary, tertiary, or quaternary cluster, ac-

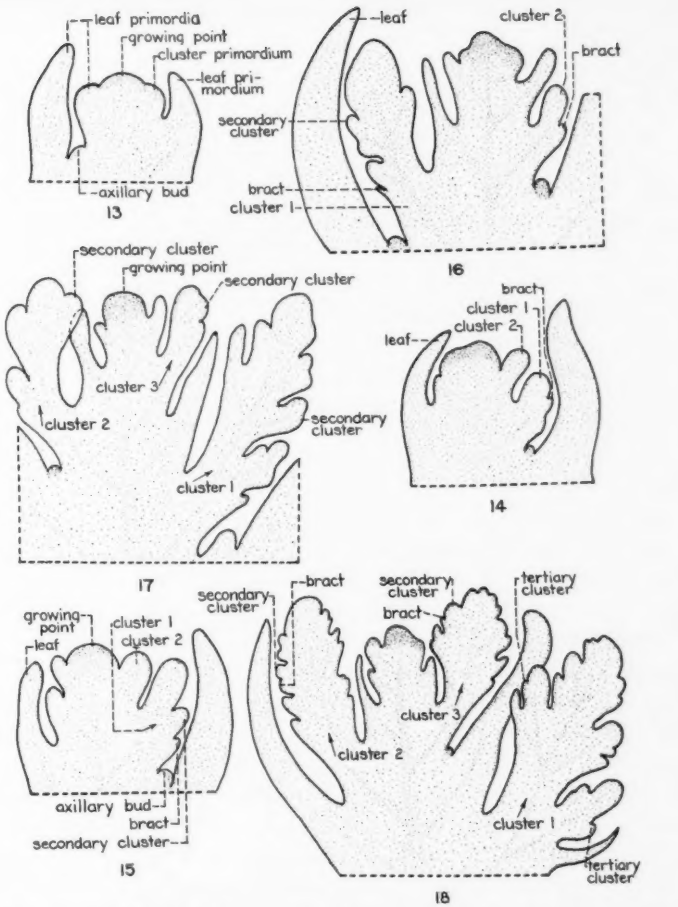
cording to the order of the axis from which it has developed. This of course is not true where the pedicel is attached directly to the rachis, inasmuch as a single fruit can hardly be considered a cluster. On account of the necessity of referring frequently to the subdivisions of the rachis, the term "cluster" is used in preference to axis, "secondary cluster" being more descriptive and convenient, for example, than "axis of the second order."

A cluster bract is first evident in the form of a small lobe, which is sometimes pointed, arising at the base of the primordial cluster (fig. 14). The appearance of an additional lobe on the main cluster marks the initiation of a secondary cluster (figs. 1, 15). The basal secondary cluster seen in figure 1 is detached from the main cluster and shown in figure 2. Each secondary cluster as well as each subsequent subcluster is subtended by a bract (figs. 1, 19, 20). Figure 3 represents the secondary cluster after it has redivided and produced three lobes. Each of these lobes originates directly from the main axis of the secondary cluster and is called a tertiary cluster (fig. 3). One or more of the tertiary clusters may subdivide to form additional lobes which become quaternary clusters. Figure 6 represents a secondary cluster in which the central tertiary cluster has undergone such division. The quaternary clusters in the basal part of the main cluster frequently undergo further subdivision when nutritional conditions are favorable.

Organogeny of flower

The first visible evidence of flower formation is a broadening and flattening of the apex of the floral axis (figs. 4, 19). The calyx appears to be a continuous ring with no indication of separate members, and instead of extending "well up" over the young bud as reported by DORSEY (5), it forms a complete cap over the bud axis (figs. 8, 9, 11). Before the cap is completely formed the petals are evident as definite lobes, and the developing petals ultimately push their way out by rupturing the calyx cap (figs. 7, 10).

As the petals elongate and come nearly into contact with one another, the cells of the apical regions enlarge rapidly, producing a roughened surface. Extensive cell division is initiated in this region, and a considerable mass of callus at the tips of the petals results.



FIGS. 13–18.—Fig. 13, bud representing shoots collected June 5. Note growing point, cluster primordium, leaf primordia, and axillary bud. Fig. 14, bud showing cluster 1 subtended by bract and cluster 2 in form of a simple protuberance, taken from middle region of shoot collected June 13. Fig. 15, bud collected June 20 with cluster 1 showing secondary cluster in axis of bract and cluster 2 in the form of simple protuberance. Growing point and an axillary bud also evident. Fig. 16, bud collected June 27 showing cluster 1 with several secondary clusters. Lobe at base of secondary cluster suggests secondary bract subtending secondary cluster. Fig. 17, bud collected July 17, in which cluster 1 shows numerous secondary clusters. Several secondary clusters are visible in cluster 2, and cluster 3 shows evidence of a secondary cluster. This is the first appearance of a third cluster. Fig. 18, bud collected April 9. In cluster 1 several secondary clusters have produced tertiary clusters. Note that in basal secondary cluster the central tertiary cluster is larger than the other two. Tertiary clusters in this condition frequently undergo further subdivision, producing quaternary clusters. Secondary clusters are numerous in each of clusters 2 and 3. Figs. 13–17 ×10; fig. 18 ×20.

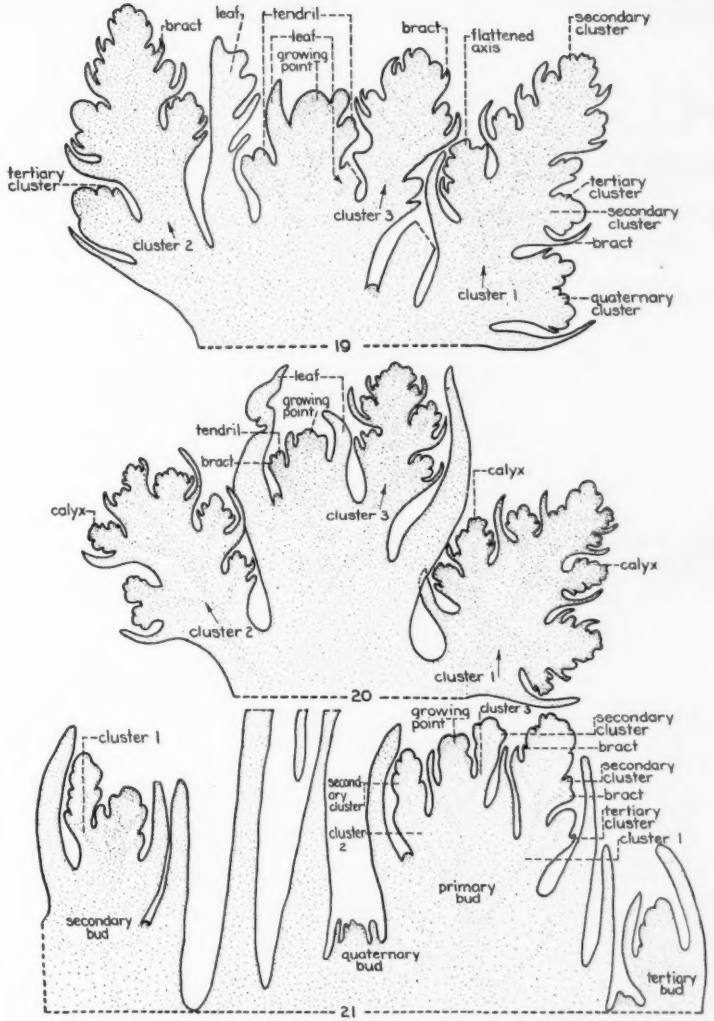
The elongating petals curve inward so that the callus tissues at the tips of opposite petals come into contact and coalesce, thereby bringing about fusion of petals in this region (figs. 9, 10). Adjacent petals, although in contact, apparently do not fuse at the base. The single cycle of stamens, arising inside and opposite the petals, is evident at about the time the petals begin to coalesce (fig. 8). The stamens show no evidence of differentiation into anther and filament at this time, but consist of large irregular cells surrounded by a single layer of small uniform epidermal cells. In cross-section the stamens are obovate, the abaxial surface being nearly in contact with the inner surface of the petals (fig. 11).

Chronological development of cluster on shoot

A flattening of the growing point resulting from extensive cell division of hypodermal cells near one side of the bud axis at this point is the first suggestion of cluster initiation. The cluster, first evident as a shoulder and then as a lateral protuberance from the bud axis, can soon be distinguished from a leaf primordium as described in a previous paragraph (fig. 13). In order to show their chronological development, drawings were made of the most advanced buds from those collections showing more advanced development than buds of preceding collections (figs. 13–21).

The first discernible cluster is evident when the shoot is approximately 1 foot long (fig. 13). Within a week after its differentiation this cluster bears a bract, immediately after which the main part of the cluster undergoes rapid subdivision, forming secondary clusters (figs. 14, 15). Further subdivision of the cluster progresses rapidly until about mid-July, when the growth of both cluster and shoot slows down. The secondary clusters continue to branch, however, so that by the end of the growing season numerous tertiary clusters are discernible in cluster 1.

Cluster 2, evident approximately one week later than cluster 1, develops according to the same plan and at about the same rate (fig. 14). Consequently the second cluster, instead of bearing numerous tertiary clusters at the beginning of the dormant period, bears numerous secondary clusters only (fig. 21). Cluster 3, in comparison with cluster 2, is evident about two weeks later and shows



FIGS. 19–21.—Fig. 19, bud showing first evidence of flower bud differentiation. Note flattened axis in one of basal secondary clusters in cluster 1. In cluster 2 the central tertiary clusters in many of secondary clusters are beginning further subdivision and form quaternary clusters (indicated by increased size compared with adjacent ones). Cluster bracts show considerable development in all three clusters. Tendrils discernible at nodes just beyond cluster 3. This drawing is taken from the the 26th bud on a shoot collected April 24. Fig. 20, bud showing first evidence of calyx, the lobes being discernible in clusters 1 and 2. In cluster 3 some of the tertiary clusters are about to undergo further subdivision. Note tendril with prominent lobes and bract at node beyond cluster 3. This is the 13th bud from shoot collected May 1. Fig. 21, grape eye showing primary, secondary, tertiary, and quaternary buds, collected September 12. Note presence of several secondary clusters in cluster 3. Fig. 19 ×20; figs. 20, 21 ×15.

evidence of one or two instead of numerous secondary clusters at the beginning of dormancy (fig. 21).

Cluster primordia are initiated in the newly forming buds in the growing region of the shoot simultaneously with development of the early buds, so that by the end of the growing season all buds on the shoot contain approximately the same number of primordial clusters. Throughout the growing season the buds in the middle region of the shoot show more advanced development than those at its extremities, although toward the end of the season there is an apparent tendency for all buds on a shoot to reach the same stage of development. This condition is realized by the beginning of the dormant period.

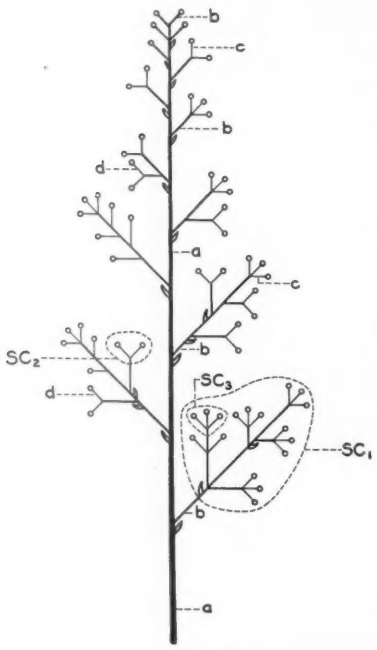


FIG. 22.—Diagrammatic drawing of grape cluster showing: *a*, main axis or rachis; *b*, axis of second order; *c*, axis of third order; *d*, axis of fourth order; SC_1, secondary cluster; SC_2, tertiary cluster; SC_3, quaternary cluster.

The growth of the cluster during the dormant period consists of a very slight enlargement of already existing subclusters. For a period of one or two weeks in the spring, before bud swelling is noticeable, the secondary clusters undergo considerable cell division and enlargement, resulting in larger and more numerous subclusters. The first visible evidence of flower formation occurs at about the time the buds begin to swell (April 24), appearing first and determinately in the basal subclusters. Differentiation of definite floral structures occurs in acropetal order immediately following the initiation of flower formation.

Summary

The data on flower bud formation in the Concord grape indicate that cluster initiation occurs in the buds of the young shoots and continues in the newly forming buds throughout the growing season. Furthermore, subdivision of the differentiated clusters begins very soon after differentiation of the clusters, and continues until the following spring, when the buds begin to swell and flower formation is evident.

The writer wishes to express his appreciation to those who aided the progress of this investigation, especially Professors B. S. PICKETT, J. E. SASS, and H. W. RICHEY, all of Iowa State College, Ames, Iowa.

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CYTOLOGICAL AND PHYLOGENETIC STUDIES IN THE CUCURBITACEAE

THOMAS W. WHITAKER

(WITH SIXTEEN FIGURES)

Introduction

The majority of investigators working with the Cucurbitaceae have confined themselves to those species which are of economic importance, although McKAY (12) has reported the chromosome number and behavior of some of the wild species. The present paper submits additional data regarding chromosome number and interspecific and varietal crossing in this group. In addition, the relationships within the family are briefly discussed.

PASSMORE (14) and McKAY (12) have fully treated the literature. The results of past work on chromosome number and the localities in which the various genera are endemic have been summarized in table I. Because the chromosomes of most members of this group are relatively small and numerous, there has been considerable controversy over the number in many of the species.

The Cucurbitaceae are distributed over most parts of the globe, with the exception of the most extreme sections of the temperate zones and the arctic regions. As a rule they are extremely sensitive to the lower temperatures, and reach their highest development in the tropics of both hemispheres. According to ENGLER and PRANTL, 54 genera are from the Old World tropics and 36 from the New, with 7 genera common to both hemispheres. The recent work of VAVILOV (16, 17) on the origin and distribution of cultivated plants seems to indicate that there are roughly three centers of origin from which the cultivated members of this family have radiated: tropical Africa; the highlands of Central America and southern Mexico; and the Asian center, located along the southwestern slopes of the Himalayas.

Investigation

The seeds for the plants used in this experimental work were secured, for the most part, from various botanic gardens through the

Brooklyn Botanic Garden, the remainder being procured from commercial seed-houses.

The results regarding chromosome numbers of the several species and varieties tested during the course of the work are recorded in

TABLE I

Genus	Chromosome number (n)	Habitat	Investigator
Melothria	12	Old World tropics	McKay
Momordica	11	Tropical Africa and Asia, 2 species in East Indies	McKay
Luffa	11–13	Asiatic tropics	McKay, Passmore
Bryonia	10	Mediterranean area	Meurman, Strasburger
Ecballium	12	Mediterranean area	McKay
Citrullus	11	Tropical Africa	Kozhukhow, Passmore, Whitaker
Cucumis	7–12	Old World tropics	Kozhukhow, McKay, Passmore, Heimlich, Whitaker
Bryonopsis	12	Africa, East Indies, Australia	McKay
Benincasa	12	Asiatic tropics	McKay
Gymnopetalum	11	East Indies	McKay
Lagenaria	11	Old World tropics	McKay, Whitaker
Trichosanthes	11	Southern Asia, East Indies, and Australia	McKay
Cucurbita	20–24	North and South America	Kozhukhow, McKay, Passmore, Castetter, Ruttle, Whitaker
Coccinia	12	Tropical Asia and Africa	McKay
Echinocystis	16	North and South America	McKay, Kirkwood
Sicyos	12	Probably Europe	McKay
Cyclanthera	16	North and South America	McKay

table II. The methods of fixing, staining, and sectioning are the same as those used formerly (**18**).

In addition to the work on chromosome numbers, the writer was interested in discovering, wherever a reasonable doubt existed, whether there was a possibility of making intergeneric or interspecific crosses in this family, and whether any of these species would cross under field conditions. In an attempt to answer this question, controlled reciprocal pollinations were made according to the scheme outlined in table III.

The varieties of the species used in making the crosses (table III)

were as follows: *Cucumis melo* var. Rocky Ford; *Cucumis sativus* var. Henderson; *Cucumis anguria* var. West India Gherkin; *Citrullus vulgaris* var. Excel; *Cucurbita pepo* var. Long Island Bush; *Cucurbita*

TABLE II

SPECIES	CHROMOSOME PAIRS
Melothria punctata Raf.	12
M. abyssinica Naud.	12
Momordica balsamina L.	11
M. charantia L.	11
Luffa cylindrica (L.) Roem.	13
Ecballium elaterium (L.) A. Rich.	12
Citrullus colocynthis (L.) Schrad.	11
Cucumis melo L. var. Golden Beauty	12
C. melo L. var. Persian	12
C. myriocarpus Naud.	12
Benincasa cerifera Savi.	12
Cucurbita melanosperma A. Br.	20
C. pepo var. Orange gourd	20
C. pepo Orange gourd×Long Island Bush	20
C. moschata var. Calhoun	20
Cyclanthera pedata Schrad.	16

TABLE III

SPECIES	POLLINATIONS	FRUIT SET
Cucumis melo×Cucumis sativus	20	None
Cucumis sativus×Cucumis anguria	20	"
Cucumis melo×Cucumis anguria	20	"
Citrullus vulgaris×Citrullus colocynthis	5*	Three
Citrullus vulgaris×Cucumis anguria	10	None
Citrullus vulgaris×Cucumis melo	10	"
Citrullus vulgaris×Cucumis sativus	10	"
Cucurbita pepo×Cucurbita moschata	20	Two
Cucurbita pepo×Cucurbita maxima	20	None
Cucurbita moschata×Cucurbita maxima	20	Two

* This figure represents the total number of pollinations. Two of the fruit resulted when *C. colocynthis* was used as the pistillate parent; the third fruit had *C. vulgaris* as its pistillate parent.

maxima var. Mammoth Chile; *Cucurbita moschata* vars. Calhoun and Japanese Chirimen.

The technique employed in making the controlled pollinations was as follows. In the case of a typical monoecious species, such as

Cucumis sativus, the staminate and pistillate flowers were isolated on the day previous to anthesis by enveloping the flower in a paper bag. On the following morning the pollinations were made and the pistillate flowers were again inclosed. After several days the bag was removed and the fruit tagged. This procedure was slightly modified for *Cucumis melo*, which is typically andromonoecious, the hermaphroditic flowers being castrated the day previous to anthesis. After this step the procedure was the same as that described for *C. sativus*.

In the three annual species of *Cucurbita*, all of which are monoecious, and in which the flowers are large, each flower was inclosed tightly by means of a rubber band around its corolla tip. The flowers were inclosed on the day previous and pollinated on the morning of anthesis. The female flowers were immediately bagged after pollination, the bag being later removed for tagging.

The results of the work with species crossing in *Cucumis* (table III) indicate that the three species under observation are all cross-sterile. Negative results were also obtained when attempts were made to cross these three species with *Citrullus vulgaris*.

Citrullus vulgaris and *C. colocynthis* are easily hybridized. *C. colocynthis* differs from *C. vulgaris* chiefly in the size of the fruit and seed. The fleshy part of the fruit characteristically has a bitter taste, but possesses the same solidity and color as some forms of *C. vulgaris*. Their vegetative characters are practically identical.

The genetic relationship of the three annual cultivated species of *Cucurbita* has been described by CASTETTER (**2**) and others. CASTETTER secured hybrid fruit by crossing *Cucurbita pepo* and *C. maxima*. This cross was successful when done reciprocally. In the case of *Cucurbita pepo*×*C. moschata* the situation was somewhat different; fruits were produced only when *C. pepo* was used as the pistillate parent and *C. moschata* as the staminate parent.

The cross *Cucurbita maxima*×*C. moschata* can be made rather easily (table III), and is successful when done reciprocally. An interesting sidelight on this cross is the fact that on the F_1 hybrid plants the staminate flowers are abortive. They are normal in every respect until approximately two or three days before the time of anthesis; then development ceases and the flowers rapidly disintegrate without opening.

During a period of over two years, not a single staminate flower of the F_1 hybrid between *C. maxima* (var. Mammoth Chile) and *C. moschata* (var. Japanese Chirimen) opened, whereas the pistillate flowers were apparently normal although not abundant. Fruit containing viable seeds was secured by back-crossing the pistillate flowers of the hybrid with the staminate flowers of one of its parents (*C. maxima* var. Mammoth Chile). A majority of the pollen mother cells of the staminate flowers of the F_1 plants never undergo the reduction division. They are differentiated from the parenchymatous tissue of the anther, enlarge to some extent, and then become disorganized. Other staminate flowers go through the usual meiotic divisions, and upon formation of tetrads, one (or sometimes two) of the cells of a tetrad fails to develop. The pollen grains of the anther sac have a shriveled appearance.

Of the three fruits obtained from the pollinations between *Cucurbita maxima* and *C. moschata*, two resulted when *C. maxima* was used as the pistillate parent and *C. moschata* as staminate parent. One of these fruits contained viable seed; the other was the result of the reciprocal cross. The seeds of this latter fruit were non-fertile, indicating possibly that the fruit was parthenocarpic in origin. The two fruits resulting from the combination *Cucurbita maxima*×*C. pepo* contained undeveloped seeds and were probably parthenocarpic.

The results recorded in table III point to the conclusion that species crossing is not a common thing, and, if occurring at all under field conditions, would involve *Cucurbita maxima* and *C. moschata* in *Cucurbita* and *Citrullus vulgaris* and *C. colocynthis* in *Citrullus*.

Discussion

The chief contribution of investigators in the Cucurbitaceae has been the recording of the chromosome numbers of several species. The striking variability of the external morphological characters of a number of the genera and species of this family apparently is not correlated with chromosome number differences. This has been demonstrated in the case of *Cucumis sativus*, in which the chromosome number of a great variety of forms, as reported by several investigators, has always been found to be $n = 7$. To determine wheth-

er there was a difference in chromosome number between the ordinary cultivated varieties of this species and some of the aberrant ones, the pollen mother cells of the varieties Lemon and Long Green were examined. In both cases the usual 7 pairs of chromosomes were found (fig. 1).

Similar varietal chromosome number differences are found also in *Cucumis melo*, in which species pollen mother cells have been examined from the ordinary melon (var. Rocky Ford), the Casaba (var. Golden Beauty), and the Persian melon. In each case the haploid chromosome number was 12 (fig. 2). The other species of *Cucumis* examined was *C. myriocarpus*. This species differs widely from *C. melo* in fruit, flower, and vegetative characters. It has 12 chromosomes as the reduced number (fig. 3), which agrees with the counts made from root-tip material by McKay (**12**).

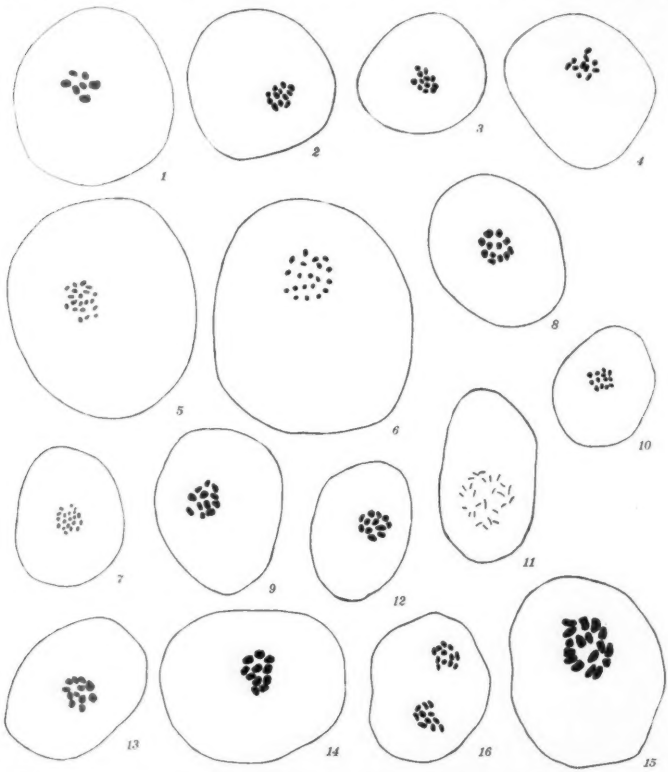
Cogniaux and Harms (**5**) have divided the genus *Citrullus* into four species, *C. vulgaris* Schrad., *C. colocynthis* (L.) Schrad., *C. naudinianus* Hook., and C. *ecirrhosus* Cogn. All these species are endemic in the Central African region. Of these four species, *C. naudinianus* and *C. ecirrhosus* are certainly distinct forms. *C. colocynthis* has generally been regarded as the wild ancestor of the cultivated variety (*C. vulgaris*). According to de Candolle (**6**), Naudin, in 1838, was able to cross these latter two species and secure fertile hybrids.

The present evidence strongly indicates that *Citrullus colocynthis* is the parent of *C. vulgaris*. Several facts point in this direction: (1) each species has 11 pairs of chromosomes; (2) they can be crossed without difficulty; (3) there is no indication that the hybrids will be sterile; (4) they are endemic in the same locality.

Pangalo (**13**) has further divided the genera of the Northern Hemisphere into four species: (a) *Citrullus aedulis*, the edible watermelon; (b) *C. colocynthis*, the colocynth; (c) *C. colocynthoides*, the citron melon; and (d) *C. fistulosus*, a small-fruited form from India. It is doubtful whether this practice is of any particular value, as these forms are all of common origin, are cross-fertile, and each has 11 pairs of chromosomes; hence there appears to be no justification for considering them as other than botanical varieties.

The small yellow-flowered gourds belonging to the genus *Cu-*

curbita are undoubtedly a form of *Cucurbita pepo*, as they readily hybridize with any of the forms of the latter and produce fertile hy-



FIGS. 1–16.—Fig. 1, *Cucumis sativus*, 1M. 7 chromosomes; fig. 2, *C. melo* var. Golden Casaba, 1M. 12 chromosomes; fig. 3, *C. myriocarpus*, 1M. 12 chromosomes; fig. 4, *Citrullus colocynthis*, 1M. 11 chromosomes; fig. 5, *Cucurbita pepo* var. Orange gourd, 1M. 20 chromosomes; fig. 6, *C. pepo* var. Orange gourd×Long Island Bush, 1M. 20 chromosomes; fig. 7, *C. melanosperma*, 1M. 20 chromosomes; fig. 8, *Benincasa hispida*, 1M. 12 chromosomes; fig. 9, *Ecballium elaterium*, 1M. 12 chromosomes; fig. 10, *Luffa cylindrica*, 1M. 13 chromosomes; fig. 11, *L. cylindrica*, 26 somatic chromosomes; fig. 12, *Melothria abyssinica*, 1M. 12 chromosomes; fig. 13, *M. punctata*, 1M. 12 chromosomes; fig. 14, *Momordica balsamina*, 1M. 11 chromosomes; fig. 15, *Cyclanthera pedata*, 1M. 16 chromosomes; fig. 16, *Momordica charantia*, 2M. 11 chromosomes; ×2400.

brids. Chromosome counts of the pollen mother cells of the Orange gourd show 20 haploid chromosomes (fig. 5). When this variety

(Orange gourd) is crossed with *C. pepo* var. Long Island Bush, the F_1 has 20 chromosomes (fig. 6).

The plants grown by the writer under the name *Cucurbita melanosperma* A. Br. correspond closely with BAILEY'S description (1) of *Cucurbita ficifolia* Bouche. This is the Malabar, or fig-leafed gourd, and it has 20 chromosomes as the reduced number (fig. 7).

The fruit, seed, and vegetative characters of the plants grown under the name *Benincasa hispida* Thunb. were identical with those figured by COGNIAUX and HARMS (5). These investigators give *B. cerifera* Savi. as a synonym for *B. hispida* Thunberg. This is the so-called white gourd, or wax gourd, cultivated for its edible fruits in China and India. The haploid chromosome number of the plants of this species was found to be 12 (fig. 8), which agrees with the chromosome number of the plants grown by MCKAY (12) under this name (*B. hispida*). The plants grown by MCKAY, and labeled *B. cerifera*, are most probably a species of *Cucurbita*, since they had 42 chromosomes.

The monotypic species *Ecballium elaterium* has 12 haploid chromosomes. The chromosomes of this species are similar to those of other species of the 24-chromosome group of the family, in that they are small and morphologically uniform.

PASSMORE (14), in a study of microsporogenesis in *Luffa cylindrica*, has figured this species with 11 pairs of chromosomes. MCKAY (11, 12) gives *L. marylandica*, *L. gigantea*, and *L. acutangula* with 13 as the haploid number and 26 as the 2n number. *L. marylandica* and *L. gigantea* are synonyms for *L. cylindrica* (COGNIAUX and HARMS 5). It is obvious that these two workers disagree on the chromosome number of the same species, *L. cylindrica*. Plants grown under the names *L. cylindrica*, *L. foetida*, and "Sooly Qua" were similar and, from all indications, belonged to the same species. *L. foetida* is a synonym for *L. cylindrica* (COGNIAUX and HARMS), while Sooly Qua is the Chinese name for the fruit of this species (STURTEVANT 9). All these plants had 13 pairs of chromosomes (fig. 10). Counts of the root-tip material of *L. cylindrica* indicate that there are 26 somatic chromosomes (fig. 11).

Plants of *Melothria punctata* Cogn. and *M. abyssinica* were identical, from all external appearances, so far as the writer's observations were concerned. COGNIAUX (4) gives *M. abyssinica* as a synonym for

M. punctata. The chromosome number was the same in both species, each having 12 as the reduced number (figs. 12, 13).

Momordica balsamina was found to have 11 haploid chromosomes (fig. 14). *M. charantia* was also found to have 11 chromosomes (fig. 16). *Cyclanthera pedata* has 16 chromosomes as the reduced number (fig. 15). The chromosomes of the latter are the largest and show the greatest degree of morphological distinction of any member of the group studied.

In dealing with the phylogeny of this family, there are a few points which it seems desirable to mention. The species that have been cultivated for their food products were all originally confined to three rather restricted areas. The regions and the species native to them are as follows: (1) tropical Africa (*Citrullus vulgaris*, *Cucumis melo*, and *Cucumis anguria*); (2) tropical India (*Luffa acutangula*, *Luffa cylindrica*, *Benincasa hispida*, *Lagenaria vulgaris*, and *Cucumis sativus;* in addition, *Cucumis melo* is found in this region); and (3) the highlands of Central America and southern Mexico (*Cucurbita pepo*, *Cucurbita moschata*, and *Cucurbita maxima*). The Old World species have basic chromosome numbers of 10, 11, 12, and 13 (with one exception, *Cucumis sativus*, which has 7 pairs of chromosomes). The *Cucurbita* species endemic in the New World have 20–24 as the basic number.

The non-cultivated members of this group coincide very closely with the cultivated members. The Old World genera have basic numbers of 10, 12, and 13 chromosomes and the non-cultivated *Cucurbita* of the New World have 20 as the basic number. In addition, there appears a New World group having 16 as the basic number, represented by *Echinocystis* and *Cyclanthera*. *Sicyos angulatus*, which has 12 as the basic number, may possibly be of American origin, but it is considered doubtful by taxonomists.

Taking into consideration the chromosome variability of the genera of the Cucurbitaceae in which the chromosome number for two or more species has been reported, we find that they apparently fall into three groups: (1) The genera in which the chromosome number of all recorded species is the same. In this classification come *Bryonia*, *Luffa*, *Momordica*, *Citrullus*, and *Cyclanthera*. In this group morphological differences apparently are not correlated with chromosome number differences. (2) *Cucumis* is placed in the second

group. There is some evidence that LEVITSKY's theory of phylogeny by chromosome fragmentation, as suggested by KOZHUKHOW (**10**), may apply to this genus. This suggestion is based on the fact that the 14 diploid chromosomes of *C. sativus* are large, compared with other members of the genus, with several showing median constrictions. These may have fragmented to form the 22 and 24 chromosome members of the genus. The evidence, however, is far from conclusive. (3) *Cucurbita* is placed in the last group. It is an example of a genus showing aneuploidy. RUTTLE (**15**), however, would place *Cucurbita* in the first grouping, as she is of the opinion that the chromosome number of all species of *Cucurbita* is 40. Several investigators have reported the diploid number of *C. moschata* as 48. Repeating their work, and in some cases using the same varieties of the latter species, RUTTLE finds 20 pairs of chromosomes.

Summary

1. The chromosome numbers of 12 species of the Cucurbitaceae have been determined from pollen mother cell material. The results may be summarized as follows: *Melothria punctata*, n = 12; *Melothria abyssinica*, n = 12; *Momordica balsamina*, n = 11; *Luffa cylindrica*, n = 13; *Ecballium elaterium*, n = 12; *Citrullus colocynthis*, n = 11; *Cucumis myriocarpus*, n = 12; *Cucumis melo* var. Golden Beauty, n = 12; *Benincasa cerifera*, n = 12; *Cucurbita melanosperma*, n = 20; *Cucurbita pepo* var. Orange gourd, n = 20; *Cyclanthera pedata*, n = 16.

2. The results of an experimental study of the fertility relations involving 4 genera and 8 species of the Cucurbitaceae indicate that species crossing is comparatively rare and not at all in accord with popular beliefs. Three species crosses were obtained: (1) between *Cucurbita moschata* and *C. maxima;* (2) between *Cucurbita pepo* and *C. moschata*; and (3) between *Citrullus vulgaris* and *C. colocynthis.*

3. The technique of making controlled pollinations among members of this family has been described.

4. The pertinent points in regard to chromosome number and geographical distribution are: (a) the Old World genera, with one exception, have basic numbers of 11–13; (b) there is a New World group having 16 as the basic number; and (c) there is a second New World group having 20–24 as the basic number.

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MYCORHIZAS OF TREES AND SHRUBS

LeRoy K. Henry

(WITH SIX FIGURES)

Introduction

The purpose of this investigation has been to determine the extent of mycorhizal infection of trees and shrubs growing in both woodland and open fields in a limited area in Butler County, Pennsylvania.

METHODS.—The smaller rootlets were carefully uncovered by means of a trowel and then traced to their respective tree or shrub by means of a spade and mattock. It is very necessary in this work to be certain of the plant from which the collection is made, and this can be accomplished only by tracing the rootlets to their point of origin from the larger lateral roots. The coralloid clusters or the swollen tips of rootlets were removed, washed in water, and placed in small bottles containing either Gilson's fixative or 5 per cent formaldehyde.

The usual laboratory methods of dehydrating, clearing, and imbedding in paraffin were employed. Sections were cut 10 μ thick, stained in Biebrich scarlet and methyl violet 6B, and mounted in balsam for permanent records. Mycorhizas were not found on every rootlet of a collection, so that often several had to be examined and sometimes a new collection of roots from the same plant had to be made the following season.

One can be rather certain of the coralloid ectotrophic types by aid of a hand lens, and even of the beaded forms of the endotrophic. In cases of uncertainty, however, it has been found that by soaking the roots in 5 per cent sodium hydroxide for three or four days and then examining under the low power of the microscope, the presence of the mycorhizas can be determined. After such treatment the ectotrophic types are readily seen as an interwoven, fungal mantle around each rootlet; but the endotrophic types show merely as small beadlike swellings. In order to determine whether these swellings

contained mycorhizal hyphae, some that had been treated with 5 per cent sodium hydroxide were imbedded. After sectioning and staining, the intracellular hyphae and granular masses were plainly visible.

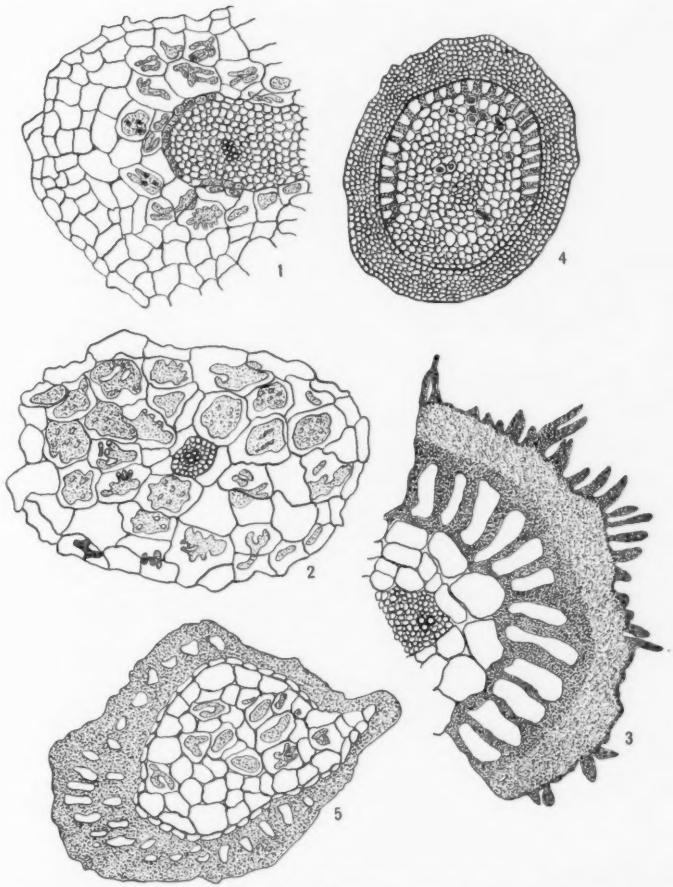
The pH value of the soil around each tree and shrub was determined by the La Motte Soil Teskit. Some rootlets were collected each month from September to June from plants growing in the woods, fields, fence rows, and yard. Mycorhizas were found on some of the collections made each month, so that it would seem that they occur throughout the year and are not restricted to woodland soils. The mycorhizas seemed most plentiful during the late fall and early winter, although good slides were often obtained from spring collections. In the winter months many dried and shriveled mycorhizas were seen, but there were always some that remained in the normal, active condition.

Types of mycorhizas investigated

Roots were collected from 60 different trees and shrubs growing in four habitats: woods, fields, fence rows, and a yard. In the districts where these mycorhizal collections were obtained, loamy, silty soils prevail which showed a pH of 5.0 beneath all of the trees and shrubs investigated. Three general types of mycorhizas were found: endotrophic, ectotrophic, and ectendotrophic. These three types were not confined to any specific kinds of soils, since they all appeared upon plants from all four of the habitats.

In endotrophic infection the hyphae penetrate the root through the root hairs and assume various intracellular forms. Individual hyphae may lie scattered or twisted about one another within the individual cell (fig. 1), or the hyphae may be woven into a skein until they appear like a mass of threads. Often the hyphae are broken into short fragments, or have the appearance of large granular intracellular masses (fig. 2) which, according to various workers as reported by RAYNER,[1] represent hyphae that are being digested by the host cells. The individual hyphae are not strictly confined to any one cell, but often extend from one root cell to another. Ex-

[1] RAYNER, M. C., Mycorhiza: An account of non-pathogenic infection by fungi in vascular plants and bryophytes. New Phytol. Reprint no. 15. Wheldon and Wesley Ltd. London, England. 1927.



FIGS. 1–5.—Fig. 1, cross-section of endotrophic mycorhiza from *Vaccinium vacillans* showing individual hyphae within the cells; ×120. Fig. 2, cross-section of endotrophic mycorhiza from *Crataegus macrosperma* showing cortical cells filled with fragments of hyphae and granular masses; ×120. Fig. 3, portion of cross-section of ectotrophic mycorhiza from *Cornus paniculata* showing prosenchyma tissue; separate hyphae project from outer surface; continuation of fungal tissue produces radial elongated cells in the cortex from inner surface; ×340. Fig. 4, cross-section of ectotrophic mycorhiza from *Pyrus coronaria* showing pseudoparenchymatous fungal mantle; ×120. Fig. 5, cross-section of mycorhiza from *Cornus paniculata* showing both ectotrophic and endotrophic infection; ×120.

ternally these infected roots are swollen at the tips and branch dichotomously, or they may appear to have beadlike constrictions borne singly or in a series.

In ectotrophic infection, the hyphae penetrate the root through the epidermis and advance intercellularly until they form a network around the cortical cells. Because of the thick mycelia between the cortical cells, the latter often become radially rectangular in shape and a thick fungal mantle replaces the epidermis (fig. 3). Often the hyphae of the fungal mantle lose their individuality and become merged into a pseudoparenchymatous tissue (fig. 4). The ectotrophic type of infection seldom progresses beyond the endodermis of the root. Roots infected with ectotrophic mycorhizas assume different external forms. There may be groups of many short roots at the tip of a larger one, giving the appearance of a cluster of corals, whence the term coralloid for this form (fig. 6); or small tubercle-like projections may be found which, when sectioned, show several roots held together by mycelia. This latter form is called tubercular. Since the formation of the fungal mat around the root prevents further growth in length, lateral roots develop which in turn are infected. It is this process that produces the coralloid clusters of ectotrophic mycorhizas.

These two types of infection are not, as was thought by an early worker as reported by RAYNER, specific upon a particular root. Often one root may show endotrophic mycorhizas and another root from the same plant may possess the ectotrophic type, or both types may be found upon the same rootlet (fig. 5). It is to this type of infection that the name ectendotrophic mycorhiza has been given. The external appearance of these roots depends entirely upon the type of their internal infection.

A transition from the endotrophic to the ectotrophic condition was observed in the case of mycorhizas from *Crataegus macrosperma*. Collections made in May showed endotrophic infection, but in October the mycorhizas were ectotrophic in external appearance. When these were sectioned and stained, many granular masses were seen within the cortical cells.

That infection is not localized at any one place on the rootlet, but arises wherever the germinating fungal spore and the root happen to come in contact, was shown by sections of mycorhizas of both types

cut in a series from the tip to 5 mm. behind it. In the ectotrophic type the mycelia occurred at the tip of the root in the form of a fungal mantle around the outside, while 2 mm. from the tip hyphae began penetrating between the cortical cells, forming the characteristic radially elongate cells.

FIG. 6.—Coralloid form of ectotrophic mycorhiza on lateral roots of *Quercus alba;* slightly enlarged.

The following list of trees and shrubs is grouped, first, according to the location of the tree with regard to ecological habitat; second, according to the types of mycorhizas found. So far as is known by the writer, the species indicated by an asterisk are new additions to those already identified as mycorhizal hosts.

Woodland

ENDOTROPHIC TYPE

*Amelanchier oblongifolia (T. & G.) Roem.
*Azalea nudiflora (L.) Torr.
*Benzoin aestivale (L.) Nees.
Carya cordiformis (Wang.) K. Koch
*Fraxinus nigra Marsh.
*Hydrangea arborescens L.
Juglans cinerea L.
Magnolia acuminata L.
*Nyssa sylvatica Marsh.
Pinus resinosa Ait.
Pinus virginiana Mill.
*Populus grandidentata Michx.
Rhus glabra L.
Tilia americana L.
Ulmus fulva Michx.
*Vaccinium vacillans Kalm.

ECTOTROPHIC TYPE

Alnus rugosa (DuRoi) Spreng.
Carpinus caroliniana Walt.
*Carya alba (L.) K. Koch
Carya glabra (Mill.) Spach.
Carya ovata (Mill.) K. Koch
Castanea dentata (Marsh.) Borkh.
Corylus americana Walt.
*Crataegus crus-galli L.
*Crataegus pruinosa (Wendl.) C. Koch
*Diervilla lonicera Mill.
*Hamamelis virginiana L.
Ostrya virginiana (Mill.) K. Koch
Pinus strobus L.
Quercus alba L.
*Quercus coccinea Muench.
Quercus velutina Lam.
Vaccinium stamineum L.
Ulmus americana L.

ECTENDOTROPHIC TYPE

*Cornus paniculata L'Her.
*Crataegus macrosperma Ashe
Fagus grandifolia Ehrh.
*Pyrus coronaria L.
Quercus rubra L.[2]
*Viburnum acerifolium L.

[2] In the fall of the year, fungi had been observed growing under trees on which mycorhizas had previously been found. Removal of excess soil showed mycorhizal rootlets matted among the dense mycelia at the base of the fungal stipe. After washing thoroughly in water and placing in a fixative, the roots still remained attached to the mycelial mass. Macroscopical investigation would seem to indicate that the fruit body of the fungus was produced by the same hyphae that form the mycorhiza. In order to verify this assumption, the base of the stipe of *Clitocybe candicans* Pers. from *Quercus rubra*, together with some of its mycorhizal rootlets, were imbedded, sectioned, and stained. These rootlets showed ectotrophic infection and the cross-sections of the stipe contained sections of roots among the mycelia.

Field

ENDOTROPHIC TYPE

Gleditsia triacanthos L.
Juglans nigra L.
*Morus alba L.
Morus rubra L.
*Rhus typhina L.
Salix nigra Marsh.
Sassafras variifolium (Salisb.) Ktze.

ECTOTROPHIC TYPE

Populus tremuloides Michx.
*Salix alba var. vitellina (L.) Koch

ECTENDOTROPHIC TYPE

Robinia pseudo-acacia L.

Fence row

ENDOTROPHIC TYPE

Acer rubrum L.
*Cornus florida L.
Liriodendron tulipifera L.
*Prunus serotina Ehrh.

ECTOTROPHIC TYPE

Fraxinus americana L.

Yard

ENDOTROPHIC TYPE

*Acer platanoides L.
Acer saccharinum L.
Picea abies (L.) Karst.
*Populus alba L.
*Sorbus americana (Marsh.) DC.

The majority of endotrophic types from plants growing in fields and in the yard possessed the typical beadlike swellings which contained fragments of hyphae and granular masses. There was an abundance of root hairs upon the normal rootlets, while among the collections from *Acer platanoides*, *Juglans nigra*, *Populus alba*, and *Sorbus americana* comparatively few roots were infected. Not all of the roots of those plants that possessed ectotrophic mycorhizas were infected, and those that were not had functional root hairs. Thus it would appear that the ectotrophic mycorhizas took the place of the root hairs, and by producing numerous coralloid clusters perhaps increased the effective absorptive area of the trees. The type, the external form, and the microscopical structure of mycorhizas on 26 new host plants are described in table I.

TABLE I

DATA FOR NEW MYCORHIZAL HOST PLANTS

NEW HOST PLANTS	DATE OF COLLECTION (1930)	HABITAT	EXTERNAL FORM OF INFECTED ROOTS	REMARKS
Endotrophic:				
Acer platanoides	May, Sept.	Y*	Beadlike swellings and swollen tips	Few roots infected
Amelanchier oblongifolia	May, Oct.	W	Swollen tips	Some sporelike bodies and granular masses within cortical cells
Azalea nudiflora	Feb., Oct.	W	" "	Granular, intracellular masses and sporelike structures
Benzoin aestivale	Sept.	W	" "	Granular, intracellular masses and sporelike structures
Cornus florida	Feb., Oct.	FR	Beadlike swellings and swollen tips	Cortical cells containing granular masses and individual hyphae
Fraxinus nigra	Sept.	W	Swollen tips	Groups of hyphal fragments
Hydrangea aborescens	Sept.	W	" "	Granular structures and sporelike bodies in cortical cells
Morus alba	May, Oct.	F	Beadlike swellings and swollen tips	Individual hyphae and hyphae in cross-section
Nyssa sylvatica	May	W	Beadlike swellings	Individual hyphae often extending from cell to cell
Populus alba	May, and Feb. (1931)	Y	Beadlike swellings and swollen tips	Majority of roots normal
Populus grandidentata	May	W	Swollen tips	Broken and individual hyphae
Prunus serotina	Feb., Oct.	R	Beadlike swellings	Intracellular hyphae and vesicle
Rhus typhina	Sept.	F	Swollen tips	Intracellular granular masses and broken hyphae
Sorbus americana	May, Nov.	Y	Beadlike swellings and swollen tips	Majority of roots normal
Vaccinium vacillans	Feb.	W	Dichotomously branched with swollen tips	Root hairs on epidermis; intracellular hyphae

* Y, yard; W, woods; FR, fence row; F, field.

TABLE I—*Continued*

NEW HOST PLANTS	DATE OF COLLECTION (1930)	HABITAT	EXTERNAL FORM OF INFECTED ROOTS	REMARKS
Ectotrophic:				
Carya alba	March	W	Coralloid	Pseudoparenchymatous fungal mantle; one row of radially elongated cells; endodermis filled with dark-staining material
Crataegus crus-galli	May, and Sept. (1931)	W	"	Fungal mantle with hyphae projecting from periphery; radially elongated cortical cells
Crataegus pruinosa	Sept. (1931)	W	"	Pseudoparenchymatous fungal mantle with radially elongated cells on one side and 3 or 4 irregular rows of cortical cells on other
Diervilla lonicera	Feb. (1931)	W	"	Few roots infected; fungal strands extending between cortical cells
Hamamelis virginiana	Oct.	W	"	Pseudoparenchymatous fungal mantle with radially elongated cells on one side only
Quercus coccinea	Feb.	W	"	Fungal mantle of interwoven hyphae; endodermis filled with dark-staining material
Salix alba var. vitellina	May	F	"	Fungal mantle of interwoven hyphae and strands between outer cortical cells
Ectendotrophic:				
Cornus paniculata	May, and Sept. (1931)	W	Coralloid and swollen tips	Fungal mantles with hyphae projecting from periphery and radially elongated cortical cells; also intracellular hyphae
Crataegus macrosperma	May, Nov.	W	Coralloid and swollen tips	Pseudoparenchymatous fungal mantles and radially elongated cortical cells; intracellular granular masses and individual hyphae
Pyrus coronaria	Oct., and Sept. (1931)	W	Coralloid and swollen tips	Thick pseudoparenchymatous fungal mantles with strands between cortical cells; intracellular granular masses
Viburnum acerifolium	May, Sept.	W	Coralloid and swollen tips	Narrow fungal mantles of interwoven hyphae; granular masses in cortical cells

Collections made in February, 1930, from a young tree of *Quercus coccinea* growing in the woods, showed ectotrophic infection. In cross-section the younger roots had fungal mantles of interwoven hyphae and radially elongated cortical cells; while the older roots had large, irregular, cortical cells with thin walls, and the endodermis was filled with a dark-staining material. Since this material was absent from the younger roots, it may be that the older roots are attempting to throw off this infection by the production of a substance which prevents the fungus from advancing. The large, thin-walled cortical cells seem to indicate a reversion to a primitive condition as a result of the infection.

Cross-sections of roots of *Hydrangea arborescens* collected in September, 1930, showed endotrophic infection with granular structures and sporelike bodies within the cortical cells. Nuclei were easily seen in those cells containing the spores, indicating that the root cells were still alive when collected.

Summary

Mycorhizas of three types, ectotrophic, endotrophic, and ectendotrophic, have been discovered on 60 different trees and shrubs, 26 of which are new additions to the list of mycorhizal host plants.

CARNEGIE MUSEUM
PITTSBURGH, PA.

PHYSIOLOGICAL VARIATIONS IN CERTAIN CROP PLANTS FOLLOWING SEED EXPOSURE TO HIGH-VOLTAGE X-RAYS

C. N. Moore and C. P. Haskins

(with six figures)

In the course of a series of investigations now in progress on the biological effects of x-rays, it was considered of interest to conduct some experiments in the subjecting of dry and moist, but ungerminated, seeds of a number of crop plants to x-rays. Considerable work has been done in this field, notably by Ancel, Goodspeed, and Stadler,[1] but it is noticeable that in most of it the radiation used was distinctly soft, the source of radiation employed in most cases being a Coolidge tungsten-target air-cooled tube operated at 50 KV and 2 to 5 MA.

It has been thought worth while, therefore, to collect certain cases of physiological abnormalities which were observed in plants arising from seeds exposed to harder radiation, both because of their striking resemblance to certain deformities occurring in *Nicotiana* species exposed as seeds to the softer rays, as reported by Goodspeed, and because certain species have not, to the knowledge of the writers, been previously subjected to x-rays, either as to sex cells, growing points, or meristematic tissues. The work was conducted entirely from the physiological standpoint, and no histological examination of resulting abnormalities was made, although it is planned to conduct such work on more promising types. Consequently, although it seems highly probable that abnormal mitoses, correlated with considerable genetic changes, are involved, no account can be given of them at the moment. It is difficult to fix the lethal dose for unger-

[1] Ancel, P., Action des faibles doses de rayons x sur des graines sèches. Compt. Rend. Soc. Biol. **91**:1435–1436. 1924.

Goodspeed, T. H., Effects of x-rays and radium on species of the genus *Nicotiana*. Jour. Heredity **20**:243–260. 1929.

Stadler, L. J., Some genetic effects of x-rays in plants. Jour. Heredity **21**:3–19. 1930.

minated seeds at any voltage, and this has not been done sufficiently clearly to be reported at this time. It is known, however, that the dosages reported here were well within the limit of tolerance in each case. For this reason, and because of scattering and differential absorption effects, it has not been possible to correlate effects with quantity of incident energy.

SOURCE OF RADIATION.—A standard Victor deep-therapy x-ray outfit was used as the source of radiation, consisting of a high-tension transformer with Snook cross-arm mechanical rectifier and a standard Coolidge water-cooled tungsten-target tube of the thick-walled type. The voltage wave form delivered by the generator was very nearly sinusoidal.

The tube was operated at 200 kilovolts peak, with 30 milliamperes current. The focal distance at which the seeds were exposed was 50 cm. The shortest wave length theoretically obtainable at such a voltage is 0.062 Ångstroms. No metal filter was used, but the glass of the thick-walled tube interposes a filtering action equivalent to about 0.10 mm. of copper. At 0.70 Å. the intensity of emergent radiation from the tube is only about 0.03 per cent of that incident on the inner surface, and this may be considered the longest wave length present in the primary radiation. The greatest intensity of radiation lay in the neighborhood of the K_{a1} line of tungsten, at about 0.21 A.U. The K_{b1} line, at about 0.18 Å., also figures prominently, and general radiation at this voltage is very considerable. Under the conditions of irradiation used, the incident dosage totaled 300 roentgen units per minute, as measured by a Victoreen dosimeter calibrated against a radium-compensated Failla ionization chamber.

TREATMENT OF SEEDS.—The seeds to be treated were divided into two portions, one of which was subjected to several hours of soaking in distilled water, followed by superficial drying with filter paper, before irradiation. Since no difference of susceptibility could be found between the dry and moist seeds at this voltage, and since this had been a usual experience with other workers using softer radiation, the distinction proved an artificial one and was eventually abandoned. Both seed lots were then spread as a single layer in containers on a table beneath the tube and given treatments varying from 0.5 to 128 minutes. Immediately thereafter the seeds were sowed in

flats or pots in good soil and propagated in a greenhouse. Some of them were later transferred to outdoor conditions, the remainder being retained under glass.

The plants illustrated represent certain of the more striking variations obtained, indicative of the type of physiological modifications most commonly met with.

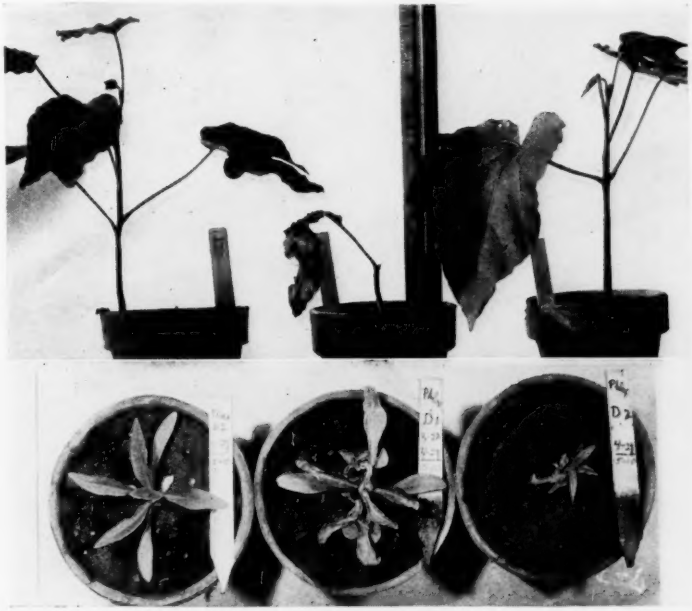
COTTON.—The cotton seeds used were of a strain of full Sea Island, kindly supplied by Dr. A. F. CAMP of the University of Florida, and exposed as described for 0.5, 2, 4, 8, 16, 32, and 64 minutes. A marked change in growth rate was evident early (fig. 1), none of the plants exposed for 32 minutes attaining more than one pair of true leaves, while extreme fasciation was evident in a large percentage of the 16-minute group. In many cases development in the last-named group proceeded from a lateral bud, the terminal bud failing to develop or doing so only to a very limited degree. Leaf form was markedly altered in a considerable percentage of the higher exposures, the changes being reflections, for the most part, of tissue duplication, inversion, and replacement (fig. 2). Complete dwarfing was not uncommon, the entire plant, including leaves, stems, and flower buds being affected so that symmetry was maintained. In such case, however, it was usual for flower development to be inhibited to the extent that the fully developed, perfect but miniature buds failed to open, passed through the typical color change from yellow to red characteristic of Sea Island, and withered while still closed. Fertilization was accomplished, however, and miniature but perfect bolls were set.

TUNG OIL.—Particular interest attached to the treatment of seeds of *Aleurites* because of their unusual bulk, and because little work has been done in this field. The changes produced with hard x-rays were of the same type as those obtaining with cotton. Fasciation, change of growth rate, chlorophyll defects, and tissue duplication, inversion, and replacement with corresponding alteration of leaf-form, were all in evidence (fig. 3). Further work with tung oil is planned.

SNAPDRAGON, PHLOX.—The modifications figured for *Phlox* (fig. 4) and *Antirrhinum* (fig. 5) were typical for a considerable proportion of the population of these plants. The first is of interest as rep-

FIGS. 1, 2.—Fig. 1 (above), Sea Island cotton at bearing age (4 months) showing progressive dwarfing, fasciation, and leaf deformity: left,—control; left center,—treated dry for 32 minutes (main development made from a lateral bud); center,—treated dry for 16 minutes; right center,—treated dry for 64 minutes (complete inhibition of development in true leaves); right,—treated dry for 64 minutes (tissue deformities in first true leaves; second pair never developed). Fig. 2 (below), leaf deformations and duplications in Sea Island cotton; control at center.

resenting an unusual case of extreme fasciation in which flower color and form has remained practically unaffected, the change being confined entirely to neighboring tissue of leaf and stalk. No further physiological modifications were noticed for *Antirrhinum* in a very



FIGS. 3, 4.—Fig. 3 (above), seedlings of *Aleurites* from x-rayed seeds at age of 3 months: left,—treated dry for 8 minutes (duplication of apical portions of leaf); center,—treated dry for 8 minutes (dwarfing and extensive leaf deformity, the latter a result of tissue replacement and inversion for the most part); right,—treated wet, 0.5 minute (equivalent to control). Fig. 4 (below), *Phlox drummondi* var. *grandiflorum*, from x-rayed seeds at age of 1.5 months: left,—treated dry for 2 minutes (equivalent to control in external appearance); center,—treated dry for 1 minute (extensive and abnormal development of lateral buds); right,—treated dry for 2 minutes (dwarfing and leaf deformation and duplication).

considerable population, but it is to be noted that the limit of tolerance of the dry seed had not been approached closely.

Alterations in growth rate, leaf form, leaf pigmentation, and plant habitus similar to those illustrated were not infrequent in a popula-

FIGS. 5, 6.—Fig. 5 (above), *Antirrhinum* from x-rayed seeds at age of 3.5 months: left,—4-minute exposure (equivalent to control); right,—2-minute exposure (dwarfing and fasciation of stems and leaves; flowers normal in size, coloration, and method of attachment). Fig. 6 (below), *Citrus aurantium* from seed x-rayed for 128 minutes at age of 7 months (extreme fasciation and inhibition of leaf development; at this age the controls of this normally rapid-growing species measured nearly 10 inches).

tion of several hundred specimens of *Phlox drummondi*, and duplication of leaf tissue was noticeable, but no alteration of flower color or form which could be attributed to x-rays was noticed.

SOUR ORANGE.—Seeds of *Citrus aurantium* were given very long exposures to the high-voltage rays in the course of a program to determine the limit of tolerance for the species. A very considerable number of aberrations were obtained among the resulting plants, most of which will require further investigation before they can be reported on. Figure 6, however, represents a rather striking and clear case of extreme fasciation without leaf deformation, since the leaves, although diminutive, are of normal form. Clearly the primordia only of the later true leaves were affected, for the plant appeared normal in the first few weeks of life. Control specimens from the same stock, propagated under similar conditions, grew vigorously, as is usual with this orange, and had attained a height of 10 inches at the time that the figured plant was photographed.

RESEARCH LABORATORY
GENERAL ELECTRIC COMPANY
SCHENECTADY, NEW YORK

PLEISTOCENE FORESTS OF CENTRAL ILLINOIS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 444

JOHN VOSS

(WITH ONE FIGURE)

The character of the plant life which prevailed during the glacial and interglacial stages is a field of promising interest, which as yet is scarcely developed. Carbonaceous soil belonging to the Sangamon interglacial and Wisconsin glacial stages is to be found in some counties of central Illinois, being sometimes exposed in stream or road cuts, or encountered in well drillings and test borings for coal. The relationship of the Sangamon and Wisconsin to other Pleistocene stages is given by LEIGHTON (4) as follows:

STAGE		SUBSTAGE
		Late Wisconsin
Fourth glacial	Wisconsin	Early and Middle Wisconsin
		Iowan — Peorian loess
Third interglacial	Sangamon	
Third glacial	Illinoian	
Second interglacial	Yarmouth	
Second glacial	Kansan	
First interglacial	Aftonian	
First glacial	Nebraskan	

Following the recession of the Illinoian ice sheet, the Illinoian till during the early part of the Sangamon epoch was weathered for a long period of time, resulting in the development of a fully mature soil profile. Toward the close of the period, the Illinoian till was covered with a loess deposit, the upper surface of which also shows a definite soil profile with leaching several feet deep. The strongly developed soil profile is ample evidence that a long period intervened between the deposition of the late Sangamon loess and that of the overlying Peorian loess. According to LEIGHTON, the Peorian loess was laid down partly while the Iowan ice was retreating and partly while the Early Wisconsin ice was advancing. In all the Pleistocene

sections here mentioned the Peorian loess is covered with thick deposits of Early Wisconsin loess.

During the Late Sangamon stage and the period of Peorian loess deposition, shallow undrained depressions were formed on the upland plains. Because of a certain degree of imperviousness of the soil and the probably cool temperate conditions, with abundant regularly distributed rainfall and high humidity, peat accumulated in these basins. This peat varies in color and texture, and often contains well preserved trunks of trees lying in a horizontal position and flattened by the pressure of the overlying material.

SAVAGE (5) and BAKER (1) base their conclusions regarding the type of climate which existed during the Pleistocene largely on the character of the macroscopic plant and animal remains found in peat and loess deposits. Since not all woods have the same resistance to decay and therefore would not all be preserved in peat, the writer undertook the study of the plant remains in this peat by employing the method of pollen analysis, now being used in tracing the succession of vegetation in postglacial peat.

Pollen grains of some plants are preserved in peat long after the plant which produced them has decayed, while other pollen grains soon decay and leave no recognizable trace. The latter statement, according to ERDTMAN (2) and SEARS (6), is especially true of *Larix* and *Juniperus*. ERDTMAN (3) states that the preservation of pollen is often governed by the condition of the bog into which it falls. If the condition is such that the pollen grains remain on the surface of the bog, resulting in exposure to the oxygen of the atmosphere and to continuous changes in humidity, this will prevent their preservation in a fossil state. If they sink to deeper levels, however, where the oxygen supply is limited, it is likely that they will be preserved. In general, pollen grains are not so abundant in the interglacial peat bogs as they are in the postglacial ones. No doubt many pollens have disintegrated since the accumulation of the Late Sangamon peat, for it is estimated that at least 60,000 years have elapsed since the time of its formation. Pollen grains are often compressed through the weight of the overlying soil, a factor which makes it difficult at times to distinguish one genus of conifers from another.

Samples were collected from the following widely separated exposures in Illinois:

1. A ravine of Richland Creek in Section 20, Town 28 North, Range 2 West, Woodford County.

2. The west bank of East Bureau Creek in Section 8, Town 16 North, Range 10 East, Bureau County.

3. A ravine of Farm Creek in Section 29, Town 26 North, Range 3 West, Tazewell County.

Of the three exposures, the last one is by far the most productive, and is probably the most notable one in the United States. In the Farm Creek cut, the buried peat is exposed for a distance of 150 feet and is 11 feet thick. The bottom of the peat stratum is 30 feet above the low-water level of the stream and the top is covered with approximately 60 feet of Early Wisconsin soil (fig. 1).

The samples were obtained by digging back 2 feet or more from the face of the exposure, in order to obtain soil which had not recently been exposed to the atmosphere. Samples were taken every inch near the top and bottom of the peat and every 6 inches in the remainder of the layer. Whenever fragments of wood were found, they were collected and later identified in the laboratory.

At first great difficulty was experienced in separating the pollen grains from the silty matter. The best results were obtained by treating the peat with 20 per cent nitric acid for at least 2 hours. The solution was then centrifuged; a small quantity was removed by means of a pipette from near the surface of the solid material at the bottom of the centrifuge tube, and placed on a microscope slide. The number of pollen grains per square centimeter (in other words, the pollen frequency of the interglacial peat) was exceptionally low as compared with the general pollen frequency of postglacial material. For that reason many slides had to be examined before at least fifty pollen grains per sample were found. Tables I–III show the pollen occurrence in the interglacial peat deposits.

The pollen analyses of the peat given in the tables show that the conifers *Abies*, *Picea*, and *Pinus* were the dominant trees during the Late Sangamon and Early Peorian stages. Since pollen grains of *Picea* outnumber the others, they are probably from the common

bog spruce, *P. mariana*. All the wood collected from the three exposures proved to be *Larix* sp.

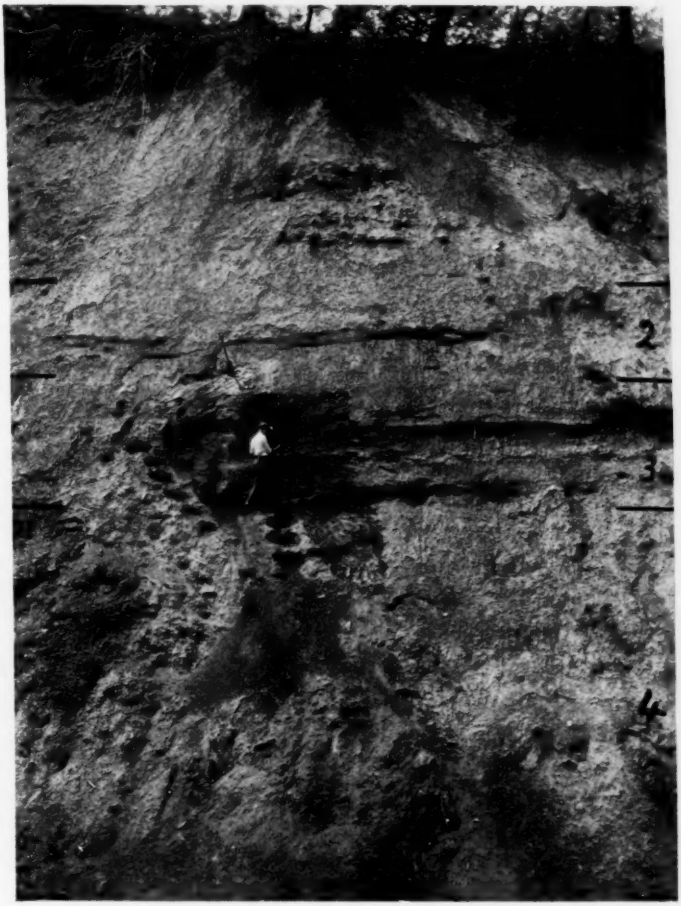


FIG. 1.—Peat exposure at Farm Creek, Tazewell County, Illinois: 1, Early Wisconsin loess, gravel and till; 2, Peorian loess, unweathered; 3, Late Sangamon peat; 4, Illinoian till, with weathered horizon.

TABLE I

POLLEN OCCURRENCE IN RICHLAND CREEK EXPOSURE

DEPTH IN INCHES	CHARACTER OF DEPOSIT	POLLEN			
		ABIES	PICEA	PINUS	POLLEN FREQUENCY
	EARLY PEORIAN				
1	Yellow clay	0	0	0	0
2	" "	0	0	0	0
3	" "	0	0	0	0
4	" "	0	0	0	0
5	Dark gray, woody, Picea leaves	7	38	12	1/2
6	" " " " " "	12	36	9	1
12	Black, woody, Picea leaves	6	41	14	2
18	Yellow clay	0	0	0	0
24	" "	0	0	0	0
30	Brown, woody, Potamogeton	7	42	4	1
36	" " "	4	44	6	1/2
42	Brown, woody, Picea leaves	6	41	6	1/2
48	Brown, few Potamogeton	3	44	2	1
54	Brown, many Potamogeton	5	42	5	1
60	Brown, woody	9	36	6	1
61	Blue clay	0	0	0	0
62	" "	0	0	0	0
63	" "	0	0	0	0
64	" "	0	0	0	0

TABLE II

POLLEN OCCURRENCE IN EAST BUREAU CREEK EXPOSURE

DEPTH IN INCHES	CHARACTER OF DEPOSIT	POLLEN			
		ABIES	PICEA	PINUS	POLLEN FREQUENCY
	EARLY PEORIAN				
1	Yellow clay	0	0	0	0
2	" "	0	0	0	0
3	" "	0	0	0	0
4	" "	0	0	0	0
5	Brown	11	38	9	1
6	"	9	43	6	1
12	Gray, slightly woody	4	48	4	2
18	Gray, woody with Picea leaves	7	45	8	2
24	Black, very woody, Potamogeton	9	41	5	3
30	" " " "	7	39	8	1
36	Black, very woody, Picea leaves	12	42	4	1/2
42	Dark red, very woody	8	43	5	1/2
48	Dark red, not woody	0	0	0	0
52	Brown	0	0	0	0
53	"	0	0	0	0
54	"	0	0	0	0

In the Farm Creek exposure the top of the Early Peorian is overlain with 5 feet of gray, fossiliferous Late Peorian loess. A thin

TABLE III

POLLEN OCCURRENCE IN FARM CREEK EXPOSURE

DEPTH IN INCHES	CHARACTER OF DEPOSIT	POLLEN			
		ABIES	PICEA	PINUS	POLLEN FREQUENCY
	EARLY PEORIAN				
1.....	Yellow clay	0	0	0	0
2.....	" "	0	0	0	0
3.....	" "	0	0	0	0
4.....	" "	0	0	0	0
5.....	Yellow clay with small pieces of wood	11	37	4	1
6.....	Dark gray, Picea leaves, Potamogeton, small twigs	9	40	6	2
12.....	Dark gray, Picea leaves, Potamogeton	13	32	8	2
18.....	" " " " "	8	39	2	1/2
24.....	" " " " "	7	47	5	1/2
30.....	" " " " "	0	0	0	0
36.....	Dark gray, wood 3–4" diameter	0	0	0	0
42.....	Light gray, very woody, many Picea leaves	13	42	6	1
48.....	Black, very woody	0	0	0	0
	LATE SANGAMON				
54.....	Black, very woody	9	45	1	2
60.....	Black, logs 4" diameter, Picea leaves	8	41	2	1/2
66.....	" " " " " "	10	47	9	1/2
72.....	Black, very woody	0	0	0	0
78.....	Gray, no wood	0	0	0	0
84.....	Light gray	0	0	0	0
90.....	Black, woody, Picea leaves	5	48	13	5
96.....	Black, no wood	7	46	15	3
102.....	Dark brown, no wood	12	41	8	7
108.....	Dark brown, few Picea leaves, Potamogeton	11	39	11	3
114.....	Black, extremely woody, Potamogeton	10	42	8	4
120.....	Black, no wood	13	47	14	5
126.....	Yellow	0	0	0	0
132.....	Dark brown	8	41	5	1/4
133.....	" "	0	0	0	0
134.....	" "	0	0	0	0
135.....	" "	0	0	0	0

humus layer was found at the top of the Late Peorian in which the following mosses were found: *Hypnum curvifolium* (Hedw.), *Calliergon giganteum* (Schimp.) Kindb., *Calliergon* sp., and two species of

Plagiothecium. Branches and roots of *Larix* sp. were found scattered throughout the Late Peorian loess, and were especially abundant just below the humus horizon.

Summary

The data seem to warrant the conclusion that forests of *Abies*, *Picea*, *Pinus*, and *Larix* existed in central Illinois for many centuries during the Late Sangamon and Early Peorian periods. Macroscopic remains also show the presence of *Larix* during the Late Peorian stage. The climate was apparently the same as that of the region in which the northern conifers are now found. This area extends in the north from Labrador and Newfoundland to northern British Columbia and the Yukon, and in the south includes the northern parts of Michigan, Wisconsin, and Minnesota.

Grateful acknowledgment is here made of my indebtedness, for valuable suggestions and advice, to Dr. GEORGE D. FULLER, of the University of Chicago, and to Dr. M. M. LEIGHTON, Chief of the Illinois State Geological Survey. To the Forest Products Laboratory, Madison, Wisconsin, to Dr. SAMUEL J. RECORD, of Yale University, to Dr. A. P. DACHNOWSKI-STOKES, of the U.S. Department of Agriculture, and to Dr. SEVILLE FLOWERS, I am indebted for the identification of the macroscopic plant remains.

MANUAL TRAINING HIGH SCHOOL
PEORIA, ILL.

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ABNORMALITY IN SWEET CHERRY BLOSSOMS AND FRUIT

G. L. PHILP

(WITH FIVE FIGURES)

Many abnormal sweet cherry (*Prunus avium*) flowers were observed during the spring of 1932. In the midseason varieties, Napoleon, Bing, and Hoskins, many flowers showed several pistils extending through the tip of the unopened blossom. Upon examination it was discovered that these protruding pistils instead of anthers occurred at the tips of the filaments (fig. 1). Figure 2 shows a normal blossom. In many cases abnormal development of the petals had also occurred (fig. 3). An occasional abnormal blossom had been observed in other years, but in 1932 so many were encountered, particularly in the Napoleon and Bing varieties, at the University Farm, Davis, California, that it was decided to investigate the extent of this abnormality.

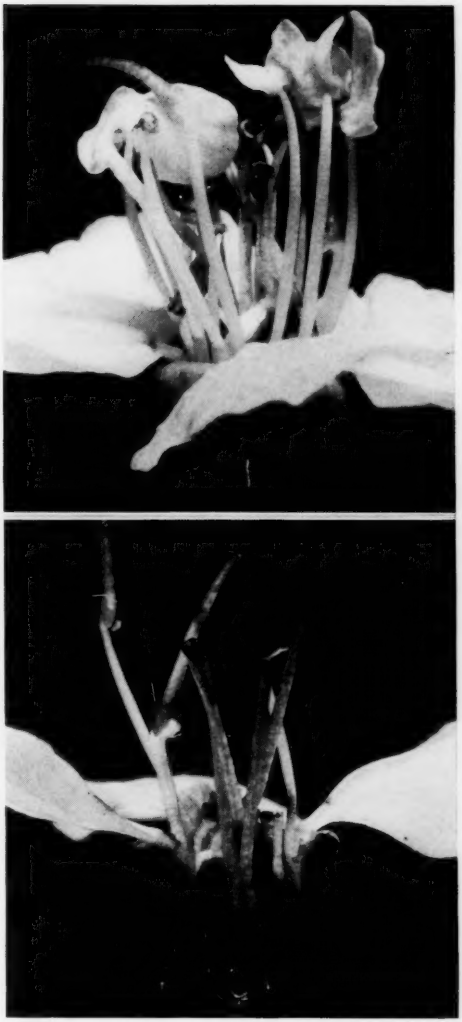
There are extensive cherry plantings in Santa Clara, Sonoma, Napa. Solano, Sutter, Placer, and San Joaquin counties. Observations were made in all of these areas for abnormal cherry flowers, and at harvest season fruit was examined. It was found that the occurrence of abnormal flowers was extensive in the hot interior valleys of the Sacramento and San Joaquin, becoming considerably less in the Sierra Nevada foothills to the east and also in the foothills to the west. No abnormal flowers were found in the coastal valleys of Santa Clara, Napa, and Sonoma, where summer temperatures are lower.

Practically all the abnormal flowers contained double pistils (fig. 4), which upon development produced double or malformed fruits (fig. 5). Fruit counts, however, indicated that many otherwise normal blossoms contained double pistils as illustrated in figure 4.

In a cherry orchard in Sutter County, a number of spurs having abnormal blossoms were tagged. All the normal flowers were removed and only flowers showing abnormal pistil or petal development were counted. No record was made as to whether or not the ab-

normal flowers contained double pistils. Table I gives the results of these counts.

FIGS. 1, 2.—Fig. 1 (above), abnormal cherry flower; note abnormal pistils attached to end of filaments. Fig. 2 (below), normal cherry blossom; *cf.* fig. 1.



FIGS. 3, 4.—Fig. 3 (above), abnormal petal development on end of filaments. Fig. 4 (below), in addition to abnormal pistil development on top of filaments, note the double pistil.

On one limb of a Bing tree 126 fruits matured, 74 of which were normal, 18 double, and 34 malformed. In 200 Napoleon fruits from this orchard (orchard run), 92 were normal, 50 were doubles, and 58 were malformed. There was a greater percentage of abnormal fruits near the ends of the branches. At harvest, counts were made of Napoleon cherries from a 10-year-old tree at the University Farm, segregated as terminal fruit (less than 3 feet from the ends of the

TABLE I

FRUIT DEVELOPMENT FROM ABNORMAL CHERRY BLOSSOMS

VARIETY	NO. OF ABNORMAL BLOSSOMS	FRUIT MATURED		
		NORMAL	DOUBLES*	MALFORMED†
Bing.....	119	0	5	11
Napoleon.....	234	1	1	18

* Double fruit: both ovaries developed equally.

† Malformed fruit: one ovary completely developed while other is only partially so (fig. 5).

branches) and inside fruit (more than 3 feet from the ends of the branches). The results are given in table II, which also gives the analysis of a composite sample of terminal and inside fruit, collected from six trees in the orchard selected at random. Table II shows that a greater percentage of doubled and malformed fruits occurs near the ends of branches than farther back on the limbs.

Table III segregates the number of normal, double, and malformed fruits from the entire crop from a Napoleon tree at the University Farm. The development of doubles and malformed fruits was not confined to the sweet cherry. While it was most prevalent with Napoleon and Bing varieties, it was also common in Montmorency (*Prunus cerasus*). The results of a composite sample from ten Montmorency trees are shown in table III.

It was generally observed that the Napoleon and Bing varieties produce many double and malformed fruits in the interior valley and none or very few in the coastal regions. It was also found that the percentage of double and malformed fruit varies considerably from year to year. Very few abnormal fruits occurred in 1931, in the in-

terior valleys, while the tables show a significant number in 1932. Temperature records at Davis show a total of 20 days in 1930 when the temperature reached 100° F. or above. In 1931 there were 30

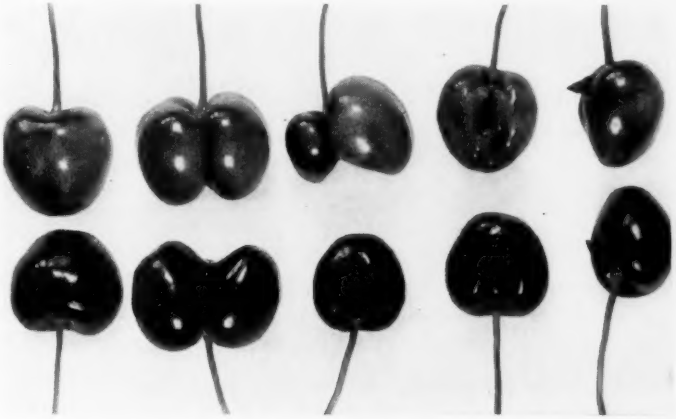


FIG. 5.—Top row, Napoleon; bottom row, Bing: normal fruit at left, from development of normal flower; other fruits showing varying degrees of development of double pistils.

TABLE II

NAPOLEON CHERRIES	NUMBER OF FRUITS					
	NORMAL		DOUBLES		MALFORMED	
	Terminal	Inside	Terminal	Inside	Terminal	Inside
From single 10-year-old tree	157	276	63	26	195	133
From six trees (composite sample)	275	377	74	46	284	169

days with a temperature of 100° F. or above; also 20 of the 30 hot days occurred during July, while only 7 days in 1930 for July were above 100° F.

Records in the Santa Clara Valley show no temperature of 100° F. for 1930 and only four days in 1931 when the thermometer reached

or exceeded this temperature. The Davis records show that the mean maximum temperatures for July and August, 1931, were 101.3 and 97.2 respectively, while for 1930 they were 94.2 and 94.4, indicating a much warmer summer for 1931 which was followed in 1932 by a significant number of abnormal blossoms and fruits.

TABLE III

SAMPLE	NUMBER OF FRUITS		
	NORMAL	DOUBLES	MALFORMED
Entire crop from a 10-year-old Napoleon tree.....	1013	216	1797
Composite sample of a Montmorency crop.....	557	85	243

Calculations by TUFTS[1] show the total heat units for July, 1931, to be 1318 while for the same month in 1930 they were 1034, which also indicates much higher temperatures in 1931. TUFTS and MORROW[2] found fruit-bud differentiation of the Napoleon cherry beginning in early July. It is probable that this abnormal development and the production of double and malformed fruits may be associated with climatic conditions, possibly high temperatures during the summer previous to blossoming, particularly during the period of fruit-bud formation.

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A SPHENOPTERID FRUCTIFICATION FROM THE PENNSYLVANIAN OF MICHIGAN[1]

CHESTER A. ARNOLD

(WITH THREE FIGURES)

In spite of the abundance of fernlike leaf impressions in the Carboniferous rocks, fertile specimens are infrequent, and it is only rarely that specimens are found sufficiently well preserved to show structure.

The fructification constituting the subject of this account was among some fossil plant material collected a few years ago by Dr. W. A. KELLY in a quarry near Corunna. The rock containing the fossils is a very fine grained, dark gray sandstone which effervesces freely when treated with hot acid. The fossiliferous strata exposed at Corunna are considered as belonging to the Saginaw formation of the Pottsville series, which occurs early in the Pennsylvanian system. Associated with the sphenopterid fructification is an abundance of leaves of *Megalopteris* and *Cordaites*, seeds of the *Samaropsis* and *Rhabdocarpus* type, and numerous other Carboniferous plants.

The specimen under consideration is a small portion of a frond measuring 17 mm. in length (fig. 1). The ultimate pinnules fork three or four times, forming short lobes which depart at acute angles from the main portion of the pinnule. Traversing the center of the pinnule is a simple vein which branches dichotomously, one branch forming the simple vein of the ultimate lobe, the other passing outward to fork again at the base of the next lobe. The lamina along the veins is continuous between the pinnules, is narrow, and varies but little in width. The pinnules are small, narrow, and scarcely more than 5 mm. long.

Since the specimen is but a small portion of a plant with small delicate pinnae, identification is very uncertain. Its affinity with the sphenopterid assemblage of Carboniferous leaf impressions is clearly shown by the narrowly attached pinnules which are wedge-

[1] Contribution from the Museum of Paleontology of the University of Michigan.

shaped (figs. 1, 2). Superficially it bears some resemblance to figures of such forms as *Sphenopteris divaricata* or *S. larischi*, but no attempt is made here to assign it to either of these or to any other species of that genus.

The fructifications are situated at the tips of the lobes of the pinnules (fig. 1). They appear in the impression as small star-shaped structures with the rays attached to a central receptacle. It is difficult to determine just how these structures are related to the pinnule

FIGS. 1, 2.—Fig. 1, fertile portion of frond; fig. 2, sterile portion of frond; $\times 2\frac{1}{3}$

lobes; whether they are attached directly to the laminated lobes or whether they are projected beyond on short filaments. There can be no error, however, in the association of these structures with the pinnules, because each star-shaped fructification is directly beyond the end of a lobe and in a few instances the midvein can be seen extending all the way to the receptacle.

The individual rays are 0.75 mm. long and vary in number from five to eight per group. They appear to be joined, or at least to touch each other for a portion of their length, but with their ends free. Superficially these rays seem individual sporangia attached to a cen-

tral point, as in *Calymmatotheca;* but a closer examination shows that a different interpretation must be applied to them. In a few favorable instances in which the lower surface of these structures can be seen they show as the spread segments of an oval body split several times lengthwise. The presence of spores in some of them indicates their sporangial nature. While living, these sporangia hung downward from a presumably horizontal lamina, and the spread appearance of the rays of the fossil was probably augmented by pressure during fossilization. This position of the unopened sporangia is

FIG. 3.—Spores obtained by maceration; 25×30 μ

also indicated by a lateral view of partially opened ones in a few instances. The spores, which appear as small amber colored objects, are approximately 25×30 μ in diameter and appear to have smooth walls (fig. 3).

In its generic affinities, this fructification conforms in essential features to *Zeilleria*. This genus was founded by KIDSTON (**3**) when he realized that STUR (**7**) had included two types of fructifications in *Calymmatotheca*. KIDSTON'S description of *Zeilleria* is as follows: "Involucres borne at the extremities of the pinnule segments, which are more or less produced to form a pedicel; in the earlier condition the involucres are globular, but at maturity they split into four lobes." In my specimen the number of segments varies from five to eight (fig. 1), but this difference hardly justifies removal from the genus when it is characteristic in so many other ways.

As originally described by STUR, *Calymmatotheca* consists of a globular sporangium which, upon maturity, splits into a number of segments after the manner just described for the Corunna specimen. RENAULT (**6**), however, and later ZEILLER (**8**) and KIDSTON (**4**), pointed out that the fructifications originally included in *Calymma-*

totheca are composed of a number of elongated sporangia arranged in a circle around a common point of attachment. Then KIDSTON (3) noted that two species included by STUR in *Calymmatotheca*, *C. avoldensis* and *C. frenzli*, together with *Sphenopteris deliculata*, to quote KIDSTON, "have an indusium which is erstwhile immature, globular, but at maturity splits into four valves." These three species were incorporated into the genus *Zeilleria*. *Calymmatotheca* belongs to the Marattiaceae, KIDSTON believed, while *Zeilleria* belongs to the Hymenophyllaceae. Another difference between the two forms which was pointed out by KIDSTON is that in *Calymmatotheca* the fructifying portions are entirely destitute of foliage leaves, whereas in *Zeilleria* the fertile pinnules differ but little from the sterile forms.

Later, after the discovery of the pteridosperms, KIDSTON concluded that the fructifications of *Zeilleria avoldensis* are seed-bearing cupules, as he claims to have observed slight protuberances where he supposed seeds were attached (5). GOTHAN (2) believes that these cupules are the containers of fernlike spores, and that *Z. avoldensis* is a true fern rather than a pteridosperm. CORSIN (1) has more recently investigated this species and maintains that it belongs to *Pecopteris*, closely resembling *P. miltoni*, which is supposed to be a fern of the marattiaceous type.

From the material at hand it is impossible to form any definite conclusions concerning either the pteridospermous or the filicinean affinities of *Zeilleria;* nor is it yet possible to demonstrate that some species might not belong to one group and some to another. This specimen is different from *Z. avoldensis* but its affinity with the genus is clear. Macerated preparations, however, do show that some of these star-shaped structures are dehisced spore cases in accordance with GOTHAN'S view. Whether these spores are those of ferns or are the microspores of a heterosporous plant (a pteridosperm) would be difficult to determine, since in no instance are both the male and female organs of a given species of a pteridosperm definitely known.

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THE LIQUID WAX OF SEEDS OF SIMMONDSIA CALIFORNICA

ROBERT A. GREENE AND E. OSBORN FOSTER

Simmondsia californica, commonly known in the southwest as "jojobe" or "jojove," is an evergreen shrub 5–15 feet high which is found principally in southern California, Baja California, and in southern Arizona. The seeds are approximately 12 mm. long and 8 mm. thick, and are covered by a thin but hard seed coat. Some time ago, several samples of the beans were submitted to this laboratory for analysis. The average of several determinations showed a content of 45.66 per cent crude fat. The shrub is somewhat hardy and has a low water requirement, so investigations were commenced to determine the nature of the oil which the bean contains and whether there might be some commercial possibilities for it.

Apparently the only study of the beans of this plant has been made by ROEHR (4), who states:

> The beans do not contain starch, glucose, tannin, alkaloids, or glucosides. Forty-eight per cent of the seed is soluble in a mixture of chloroform and ethyl ether. This was composed chiefly of fat and an oil of sp. grav. 0.9625 (16° C.). The oil was soluble in the usual fat solvents. The fat (42.87%) melted at 36° C., and had a faint bland odor which resembled oil of theobroma, which was bland to the taste at first, then nauseous. The seeds contain a purging principle which was not isolated, but which might be a toxalbumin similar to ricin.

Approximately 100 pounds of the seeds were received through the courtesy of Mr. F. J. CRIDER, Director of the Boyce Thompson Southwestern Arboretum, Superior, Arizona. The seeds were ground in a power mill, the oil extracted by means of a hydraulic press (300 kg. per sq. cm.), and filtered through a filter on a steam funnel. The yield was approximately 25–30 per cent by weight.

The expressed oil, which was transparent, a golden yellow color, and tasteless, was analyzed, employing the methods of the Association of Official Agricultural Chemists (1). The results were as follows:

Specific gravity 25° C.	0.8635
Refractive index (Abbé) (25° C.)	1.4650
Iodine no. (Hanus)	88.40
Reichert-Meissl no.	0.70
Polenske no.	0.31
Acid value	0.23
Soluble acids (as butyric)	2.43%
Insoluble acids	59.43%
Unsaponifiable residue	37.62%
Saponification no.	95.00
Acetyl no.	6.80

The results obtained were compared with the characteristics of various oils as given by LEWKOWITSCH (2). It was found that, with the exception of the value for the saponification number, the constants of this oil are practically identical with those of sperm oil and arctic sperm oil, which are liquid waxes.

The oil of *Simmondsia californica* is soluble in ether, petroleum ether, chloroform, carbon tetrachloride, carbon disulphide, and benzene; but is immiscible with 95 per cent alcohol, absolute alcohol, and acetone. The oil solidifies at 10°–12° C. A small amount of it was exposed to the air on a glass plate for several weeks without any evidence of drying.

Early in this study it was realized that this oil had the characteristics of a wax rather than an oil. Consequently a quantity of it was saponified with alcoholic KOH. A homogeneous solution resulted, which separated into two layers when diluted with water. The mixture was acidified in order to liberate the fatty acids, filtered, and the filtrate neutralized with NaOH, concentrated on a steam bath and tested for glycerol. The acrolein test and various color tests were doubtful, indicating that if glycerol is present, it occurs in extremely small amounts.

Another portion of the oil was saponified, diluted with distilled water, and the unsaponifiable material separated by means of a separatory funnel. Qualitative tests, such as employed in organic qualitative analysis, showed the unsaponifiable material to consist chiefly of an alcohol. This alcohol melted at 7° C. and boiled at

231° C. This would indicate that the material might be decyl alcohol. When the oil was subjected to the elaidin test, a solid isomer formed within 15 minutes. Although most waxes usually consist of the fatty acid esters of higher monohydroxy alcohols (cetyl, octodecyl, ceryl alcohol, etc.), RICHTER (3) states that n-hexyl alcohol occurs as acetic and butyric esters in the oil to the seed of *Heracleum giganteum*, and n-octyl alcohol as the acetic acid ester in the volatile oil of the same plant, and as the butyric ester in the oil of *Pastinaca sativa*. Accordingly the presence of decyl alcohol in the seeds of *Simmondsia californica* seems probable.

Sperm oil is a valuable lubricant, and because of the similarity in characteristics, this wax might therefore have some value for lubricating purposes.

Summary

1. The seeds of *Simmondsia californica* contain a liquid wax, which has been tested, and its characteristics and constants agree very closely with those of sperm oil and arctic sperm oil.

2. Qualitative tests indicate that the wax might consist principally of fatty acid esters of decyl alcohol.

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TUCSON, ARIZ.

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CURRENT LITERATURE

BOOK REVIEWS

Cycads

For many years SCHUSTER has been studying the cycads, especially from the taxonomic standpoint. He has now given a helpful account[1] of the present status of the classification, geographic distribution, and paleontological development of the group.

The known fossils are described critically and some are allowed a place in the cycadophyte phylum; some are thought to be ferns, and it is suggested that some are even palms or other angiosperms. In the case of some which are given a place in the cycadophytes, the author believes the evidence is not sufficient to determine whether the forms should be assigned to the Bennettitales or the Cycadales. Little importance is given to leaf characters in distinguishing between these two orders. The Mesozoic *Pterophyllum, Dioonites, Otozamites, Ptilophyllum*, and *Podozamites* appear in the Triassic (Rhaetic), only *Pterophyllum* going back to the Carboniferous, and they became extinct in the middle Cretaceous. *Zamites, Anamozamites, Glossozamites, Nilssonia*, and *Cycadites* became extinct in the middle Jurassic. A large part of these forms doubtless belonged to the Bennettitales, which extend from the Keuper to the upper Jurassic; but megasporophylls (*Cycadospadix* in the Rhaetic and *Zamioidea* in the Keuper) show that the Cycadaceae were also present. The Bennettitales and Cycadales are parallel developments from the Paleozoic Cycadofilicales.

In geographic distribution the Mesozoic cycadophytes were cosmopolitan, from Greenland, Alaska, and Siberia to Patagonia, New Zealand, and South Africa and all the way around, a striking contrast to the present condition, with the Bennettitales entirely extinct and the Cycadales confined to tropical and subtropical regions.

SCHUSTER thinks that the origin of the recent genera cannot be determined by the paleontological evidence, but must be conjectured from geographical and geological evidence. The reviewer would agree that little dependence can be placed upon leaves as a criterion for distinguishing between Bennettitales and Cycadales; but, for that very reason, many of the leaves assigned to Bennettitales may belong to Cycadales. Besides, if the fructifications of the ancient Cycadales decayed early, like those of the living forms, preservation would be

[1] SCHUSTER, JULIUS, Über das Verhältnis der systematischen Gliederung, der geographischen Verbreitung und der pälaontologischen Entwickelung der Cycadaceen. Ein Beitrag zur historischen Biologie. ENGLER'S Botanische Jahrbücher **64**: 165–260. pls. 4–11. 1931.

rarer than in the better protected fructifications of the Bennettitales. An occasional sporophyll, like *Cycadospadix hennoquei*, is well preserved, and it seems to differ less from sporophylls of the living *Cycas revoluta* than sporophylls within this genus differ from each other. The rapid decay of fructifications and the assigning of leaves to Bennettitales may account, in some degree, for the incompleteness of the fossil record of Cycadales.

SCHUSTER says that when we speak of a cosmopolitan distribution of the cycadophyte phylum we refer to the Bennettitales, not to the Cycadales, since the cycads in the beginning, as now, were scantily represented. Their ancestors were transitional forms between the Cycadofilicales and the modern Cycadales, arising in the upper Triassic as climatic reactions to the aridity of the Eurasian continent. These ancient forms gave off a branch to Gondwanaland and one to North America. From the descendants of these the present-day cycads arose from genetic centers in Himalaya, West Australia, South Africa, Mexico, and Central America.

The earliest trace of the Cycadaceae is found in the upper Triassic (Keuper), with *Cycadospadix*, the forerunner of *Cycas* and *Beania*, of *Zamia*, both Eurasian. Only one species of the living cycads has been found as a fossil, a species of *Cycas* in the older Tertiary of Japan. In the upper Triassic, a branch of the Zamoideae reached North America. The Permian *Autunia* and *Cycadoxylon* show that the family has come from the Cycadofilicales.

The living genera of cycads are parallel developments, none having been derived from any other living forms. *Cycas* and the American genera had a north continental origin; while the African and Australian genera, with the exception of *Cycas*, were southern. Neither the Bennettitales nor the Cycadales gave rise to the angiosperms.

The paper contains an immense amount of information, critically organized, and references to literature are adequate; but such an extensive bibliography would have been more conveniently arranged chronologically or alphabetically at the end of the contribution.—C. J. CHAMBERLAIN.

Nature's gardens and man's gardens

A number of recent books serve to emphasize the social nature of plants, whether in natural communities or in the gardens planned and planted by man. A volume of this type by WELLS describes in simple terms the plants and plant communities of North Carolina.[2] The material is organized under two general headings, The natural gardens of North Carolina, and The herbaceous wild flowers of the natural gardens. The first part consists of descriptions of the principal plant communities of the state, the second of descriptions of the chief individual plant species.

The ecological descriptions are in non-technical language that may at times be criticized as teleological. The facts presented in the discussion, however,

[2] WELLS, B. W., The natural gardens of North Carolina. pp. 458. figs. 209. University of North Carolina Press. Chapel Hill, N. C. 1932. $3.50.

seem to be well selected, accurately stated, and calculated to enable the lay reader to enjoy the landscapes of grassland, forest, and mountain with a more intelligent interest. This interest will be increased by the 123 illustrations in the first part of the book, which covers 208 pages.

In the second section are found keys and descriptions of individual plant species. The seven keys are for the plants of seven great community types. They are for the herbaceous plants of: great forest uplands, sand hills, coastal dunes, savannas, fresh-water marshes, salt marshes, and aquatic plants. In the keys, flower color and leaf form play an important part, and the general scheme seems a promising one. The descriptions of individual species are brief and simple, but aided by the numerous illustrations, will be found useful.

The year has also had an abundance of books about plants that are grown in gardens. This is particularly true of a group from the house of Macmillan. The first is a book describing the plants of alpine communities that provide the materials for the rock gardens of the lowlands.[3] The author deals with the mountains of the west, including Rainier, Hood, and the neighboring peaks and ranges. Perhaps the best part of the book is the remarkable collection of over 100 photographs of alpine plants and flowers from these mountains. There are, however, descriptions of the plants and directions for growing them in rock gardens.

A third volume is by one[4] who had two extensive Florida gardens, which were really small arboretums, including all the tropical species found in the state, together with many from other lands. He knew these plants intimately; their names, classification, requirements, habits, and beauty, and wrote about them from his rich store of information. His descriptions are sympathetic but also scientifically accurate. The gardener has now passed away, but his writings have been collected and put into a book of fascinating interest by two of his friends.

The next in the series of garden books[5] has a very different scope. It is by an English writer, who has visited the natural and cultivated gardens of America, from Vancouver to California, Arizona, Louisiana, South Carolina, Maryland, and Maine. It is the story of a garden pilgrimage charmingly told, in spite of the occasional misuse of scientific names.

In the first part of another book[6] the reader may find a vast amount of plant

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[3] GABRIELSON, IRA N., Western American alpines. pp. xviii+271. figs. 121. Macmillan Co. New York. 1932. $3.50.

[4] NEHRLING, HENRY, The plant world of Florida. From published manuscripts collected and edited by ALFRED and ELIZABETH KAY. pp. xiii+304. pls. 27. Macmillan Co. New York. 1933. $3.50.

[5] CRAN, MARION, Gardens in America. pp. 320. pls. 16. Macmillan Co. New York. 1932. $3.50.

[6] FOX, HELEN M., Gardening with herbs for flavor and fragrance. pp. xiv+334. pls. 12. Macmillan Co. New York. 1933. $3.50.

lore, together with much historical information regarding plants used for their flavors or their odors. As most aromatic plants have been regarded as possessing medicinal properties, the old "herbals" and the "doctrine of signatures" abound in curious statements about the mythical qualities of herbs. The author has drawn much from these sources. The herb lore of the United States is shown to be a mixture of that imported from Europe and that found among the North American Indians.

In the second part of the book the author describes some 70 herbs, giving for each the scientific name, a short description, its history and legend, its uses, and its culture. Several of the plants are illustrated by drawings that are artistically pleasing but would be of little assistance in the recognition of the plants. There are ten pages of bibliography.

Similar to the last, but covering a much wider field, WILDER[7] has drawn from personal experience, from catalogues, from old herbals, and from a variety of other sources a most extensive list of fragrant plants. These are described as to their botanical classification, their place in gardens, and their native homes. They are segregated into groups according to their odors and discussed on this basis.

This book too has its bibliography, but the author has drawn from poetry and history as well as from textbooks and scientific journals. An extensive index of both common and scientific names makes its information easy of access. It will make a valuable addition to our shelf of garden books.—G. D. FULLER.

Handbook of plant analysis

Earlier volumes of the handbook of plant analysis by KLEIN[8] have been noted in this journal.[9] The third volume comes in two large tomes with consecutive paging. The first half contains 806 pages, and the second 807.

The first half of volume III considers a few large groups of substances: membrane substances; naturally occurring tannins; lichen substances (acids, fats, etc.); ethereal oils; caoutchouc and gutta-percha; and resins. The second half is divided into a greater number of sections, but the substances considered really fall into two large groups, glucosides and plant pigments. Among the glucosides the following are included: glucosides with aliphatic and aromatic substances; flavones and related compounds; anthocyanins; anthracene glucosides; hydrocyanic acid glucosides; indoxyl glucosides; garlic and mustard oils and their glucosides; saponins; digitalis glucosides; and glucosides of little understood composition. The pigments considered are the carotinoids, chlorophyll, algal pigments, bacterial and fungal pigments, and other pigments which have

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[7] WILDER, LOUISE B., The fragrant path. pp. xv+407. Colored frontispiece. Macmillan Co. New York. 1932. $3.00.

[8] KLEIN, G., Handbuch der Pflanzenanalyse. III/1, 8vo. 1–806; III/2, 8vo. 807–1613. J. Springer. Vienna. 1932.

[9] BOT. GAZ. **92**:332–333. 1931; *Ibid.* **94**:427–428. 1932.

not been thoroughly investigated, such as rottlerin, santalin, vitexin, gossipol, trifolitin, etc.

The methods of detection, quantitative estimation, and preparation methods are usually given, and the occurrence and systematic distribution of the various substances in the plant kingdom are given for most of the substances included. The vast amount of information contained in these volumes is indicated by the fact that the index alone occupies 137 pages.

Generous space is given to the consideration of each group of substances. Thus in the first part of the volume, the membrane substances, cellulose, hemicelluloses, gums, mucilages, chitin, pectins, lignin, cork, cuticle, crude fiber, and phytomelane; and the membranes of algae, bacteria, fungi, mosses, and ferns; and fossil plant membranes, occupy 344 pages. Numerous literature lists are provided, with citations of recent literature. Brief sketches of the background and development of our knowledge give the work the illuminating historical perspective so much needed in current research.

The editor and publishers have provided an indispensable handbook for the use of plant chemists, plant physiologists, and all investigators whose work makes necessary a study of the products of plant metabolism.—C. A. SHULL.

Root nodule bacteria

It is fortunate for botanists that FRED[10] and his associates have summarized their work and given it setting in a monographic treatise on the root nodule bacteria and leguminous plants. This monograph contains 13 chapters devoted to: the history of Leguminosae in agriculture; general description of the Leguminosae; their distribution and importance; the occurrence of root nodules; isolation and study of root nodule bacteria; their morphology and life cycle; their cultural and biochemical characteristics; factors which influence growth and longevity of nodule bacteria; their species relationships; formation of nodules, their histology and cytology; relationship between leguminous plants and bacteria; factors influencing nodule production; economic importance of leguminous crops; natural and artificial inoculation. An appendix deals with practical problems such as use, distribution, inspection, and testing of artificial cultures. The bibliography consists of almost 60 pages of citations. The plates are excellent; there is a good index; and also a frontispiece with a portrait of HERMAN HELLRIEGEL, and a dedication to the late WILLIAM HARMON WRIGHT.

The subject matter of this field has been of continuous practical interest to man since his first attempts at agriculture. It is full of meaning for the microbiologist, the ecologist, the pathologist, the physiologist, the morphologist, the histologist, and the cytologist. It has many theoretical implications, especially for those interested in the fundamental problems of infection, pathogenesis, aggressivity and virulence, susceptibility and resistance; cellular, tissue, and organ re-

[10] FRED, E. B., BALDWIN, I. L., and MCCOY, E., Root nodule bacteria and leguminous plants. Univ. of Wisconsin Studies in Science, no. 5. pp. xxii+342. pls. 46. Madison, Wisconsin. 1932.

sponses, as well as responses of the plant as a whole to non-pathogenic and pathogenic infection. The reader will find data bearing on all of these points. He will find that while much is known, much more still remains to be discovered. It is one of the merits of this treatise that while it ably and clearly presents the known, it also deliberately calls attention to the unknown. It is obvious that ascent of the next range of peaks involves many difficulties and much preliminary trail-making of a biochemical and cytological nature.—G. K. K. LINK.

Methods

The fifth edition of the text on methods by CHAMBERLAIN[11] has been almost entirely rewritten, with new illustrations. Many things are presented for the first time. Some obsolete methods are omitted; others are retained because of historical interest.

In part I are chapters devoted to apparatus, reagents, stains and staining, a concise history of staining (including theories and practical hints on staining), temporary mounts and microchemical tests, freehand sections. The glycerin method, although practically obsolete, is described because there are some forms which still must be mounted in media with a glycerin base. The Venetian turpentine method is presented with concise and workable directions. The paraffin method is given the thorough treatment commensurate with its importance in modern technique. The now almost obsolete celloidin method is described because of its historical interest. The chapter on special methods gives those worked out by the author as well as those developed by research workers in very special fields. The chapter on paleobotanical technique gives the latest methods in use. The chapter on botanical photography is the result of the author's wide experience in this subject, extending over many years. The portion of this chapter written by PAUL J. SEDGWICK, entitled Movie Photomicrographs, should enable any one familiar with ordinary photographic methods to make successfully motion picture photomicrographs. The new chapter describing how to prepare illustrations for publication is most timely.

In part II the general principles given in part I are made specific for all the great groups of plants, since the author realizes the difficulty which beginners especially have in applying general principles to concrete cases. This part is illustrated by excellent illustrations which should serve as models for future illustrations. It is a far better textbook of botany than most of the current so-called textbooks.

The book is the result of more than 40 years of intensive study and first-hand contact with botany; and in the opinion of the reviewer, is the last word on modern botanical technique.—W. J. G. LAND.

Plants of the Rocky Mountain National Park

During the past decade the National Park Service has made a decided effort to promote the study of the vegetation of the various parks within its system.

[11] CHAMBERLAIN, C. J., Methods in plant histology. 5th ed. pp. 416. University of Chicago Press. 1932.

The Rocky Mountain National Park is one of the best known and most accessible, being visited by a quarter of million persons each year. The vegetation of this park is remarkable for its diversity and for the richness of its alpine flora. A description of its plants by ASHTON[12] cannot but make them better known to the visiting public. With its many illustrations from photographs, botanists may get much assistance upon a first visit to the mountains. The book has an ecological introduction, keys for identification, and descriptions of all the principal species given in simple but accurate language, with both scientific and common names. The latter seem to have been happily chosen so as to lead to as little confusion as possible. Most of the many photographs with which the book is illustrated are of good quality and will assist in the recognition of the plants.—G. D. FULLER.

Plant lore of Palestine

Coming from the pen of one who had spent her life in Palestine and had an intimate acquaintance with its people, this book[13] brings a strange mixture of Jewish, Moslem, and Christian truth and legend. Most of these legends cluster about useful plants such as cereals, pulse, the vine, and the olive, although medicinal plants, sacred trees, and magical plants are also included. Each plant is designated by its scientific, common English, and Arabic name, the last transliterated from the Arabic alphabet. The use made of the plant by the common people is then given, and all the mystic lore, legend, and poetry that have collected around it are recounted. There seems every reason to suppose that this book presents the true lore of the East, and as such its pages will be a source of much information concerning biblical lands.

The illustrations contain many drawings of the more common plants, affording a good idea of their appearance.—G. D. FULLER.

Potato leaf roll

Although potato leaf roll seems to have been responsible for potato failures in Europe in the last quarter of the 18th century, it was not recognized as a specific disease of the potato until 1905 and was not found in the United States until 1911. The name "Blattrollkrankheit" was first applied by APPEL in 1906. Since then the literature has become so voluminous that there has been much need of a monographic treatment of the subject.

This need has been met by the recent monograph of ESMARCH.[14] After a brief review of the history, geographical distribution, and economic importance of leaf roll, he discusses the disease thoroughly under the headings (1) symptoms

[12] ASHTON, RUTH E., Plants of Rocky Mountain National Park. pp. iv+157. pls. 15. figs. 100. National Park Service, U.S. Dept. Interior. Washington, D.C. 1933.

[13] CROWFOOT, GRACE M., and BALDENSPERGER, LOUISE, From cedar to hyssop: A study in the folklore of plants in Palestine. pp. viii+196. pls. 76. The Sheldon Press. London; Macmillan Company. New York. 1932. $2.00.

[14] ESMARCH, F., Die Blattrollkrankheit der Kartoffel. Julius Springer, Berlin. pp. 91. 1932.

and development of the disease, (2) histology, (3) physiology, (4) transmission, (5) influence of external factors, (6) internal disease factors, (7) etiology, and (8) resistance. The question of phloem necrosis is extensively discussed, the author having previously published on this subject as well as on the question of metabolism in plants affected with leaf roll. In the present monograph seven pages are devoted to the carbohydrate metabolism in diseased plants and two pages to protein metabolism, on which less is known. The monograph wisely deals only with publications occurring since 1911, literature previous to this time having been well summarized by APPEL (1911) and HIMMELBAUR (1912).—ALICE ALLEN BAILEY.

Statistical methods for research workers

The monograph by FISHER[15] has appeared in a fourth edition. It is a very useful work for any biologist who must test the dependability of results by statistical procedures. The methods presented by FISHER for such work have the advantage that they are suitable for use with few samples. This will be appreciated, since it is often difficult to provide large numbers of samples.

The main additions to the work deal with covariance. The part played by covariance is emphasized mainly in chapter V, and a section on the analysis of covariance is added to chapter VIII. There are a few minor changes in chapter III on Distributions, and the appendix to this chapter has been entirely rewritten. As a whole the changes are too few to destroy the value of the previous edition of 1930 for those who have a copy of it.—C. A. SHULL.

Flora of South Dakota

A decided want has been met by OVER[16] in compiling a check list of the vascular plants of South Dakota. The state presents a great variety of habitats, ranging from semidesert to mesophytic forests. Most unique of all are the Black Hills with their outliers from the Rocky Mountains and from the northern conifer forests. Although the present list gives little except the names of the species and the locations within the state where they are usually found, it will serve as a useful guide to future students. The illustrations are mostly halftones from good photographs; but they are arranged in a rather disorderly fashion, without consecutive numbering and with curiously varied legends, so that their usefulness is much lessened. The terminology of RYDBERG's manual has been followed.—G. D. FULLER.

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[15] FISHER, R. A., Statistical methods for research workers. 8vo. pp. xvi+307. Oliver and Boyd. Edinburgh & London. 1932.

[16] OVER, WM. H., Flora of South Dakota. pp. 161. figs. 75. University of South Dakota. Vermilion, S.D. $1.00.

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